dogs, and humans. Cysts in humans are most common in the subcutaneous tissues, eye and the brain.

Foods associated with illness include: raw or undercooked pork.

Toxoplasma gondii is a protozoan parasite that encysts in the tissues of a variety of mammalian hosts including pigs. Human infection may result in ''flu like'' symptoms in adults, late term abortions in pregnant women or serious congenial infections in children.

Foods associated with illness include: raw or undercooked pork.

Balantidium coli is a protozoal organism.

Foods associated with illness include: raw, undercooked pork (fecal contamination)

Cryptosporidium spp.

Foods associated with illness include: inadequately treated water, raw or undercooked veal or beef.

Chemical Hazards

While biological hazards are of great concern because contaminated foods can cause widespread illness outbreaks, chemical hazards may also cause foodborne illnesses, although generally affecting fewer people.

Chemical hazards can originate from four general sources:

(1) Agriculture chemicals: pesticides, herbicides, animal drugs, fertilizers, etc.

(2) Plant chemicals: cleaners, sanitizers, oils, lubricants, paints, pesticides, etc.

(3) Naturally-occurring toxicants: products of plant, animal, or microbial metabolisms such as aflatoxins, etc.

(4) Food chemicals: preservatives, acids, food additives, sulfiting agents, processing aids, etc.

(5) Environmental contaminants: lead, cadmium, mercury, arsenic, PCBs.

For many years the Food Safety and Inspection Service has conducted a National Residue Program to monitor the occurrence of residues from hazardous chemicals in meat and poultry products. Under a HACCP regime, frontline responsibility for control of residues from animal drugs or environmental contaminants will move from the government to the industry, although the agency will continue to verify that these controls and preventive measures are effective. Companies that slaughter livestock and poultry will probably find the FSIS National Residue Program Plan to be a useful document. The plan contains lists of compounds that might leave residues in the tissues of animals or birds, and provides some information on their relative risk through the rankings in the Compound Evaluation System. It provides information on which compounds FSIS has included in its annual testing program. It also provides information on the methods that are used to test for the compounds. Another FSIS document, the Domestic Residue Data Book, presents the results of FSIS testing. These data can help a HACCP team understand the overall hazard presented by various residues, although each company should gather information about the residue control performance of its own suppliers.

Another useful reference about hazardous chemicals is the FSIS List of Proprietary Substances and Nonfood Compounds. This publication lists substances used in the preparation of product and nonfood compounds used in the plant environment that have been authorized by FSIS.

Table 2 identifies some additional sources of chemical hazards. References listed in Section VIII can be used by the HACCP team in evaluating the potential chemical hazards associated with their product or process.

TABLE 2.—TYPES OF CHEMICAL HAZARDS

Location	Hazard
Raw Materials	Pesticides, antibiotics, hormones, toxins, fertilizers, fungicides, heavy metals, PCBs. Color additives, inks, indirect additives, packaging materials.
Processing	Direct food additives—preservatives (nitrite), flavor enhancers, color additives. Indirect food additives—boiler water additives, peeling aids, defoaming agents.
Building and Equipment Maintenance	Lubricants, paints, coatings.
Sanitation	Pesticides, cleaners, sanitizers.
Storage and Shipping	All types of chemicals, cross contamination.

Physical Hazards

Physical hazards include a variety of materials referred to as extraneous materials or foreign particles or objects. A physical hazard can be defined as any physical material not normally found in a food that can cause illness or injury to a person consuming the product.

Physical hazards in finished products can arise from several sources, such as contaminated raw materials, poorly designed or maintained facilities and equipment, faulty procedures during processing, and improper employee training and practices. Table 3 identifies some common physical hazards and their causes or sources.

TABLE 3.—TYPES OF PHYSICAL HAZARDS

Hazard	Source or cause
Glass	Bottles, jars, light fixtures, utensils, gauge covers, thermometers.
Metal	Nuts, bolts, screws, steel wool, wire, meat hooks.
Stones	Raw materials.
Plastics	Packaging materials, raw materials.
Bone	Raw material, improper plant processing.
Bullet/BB Shot/Needles	Animals shot in field, hypodermic needles used for infections.
Jewelry	Pens/pencils, buttons, careless employee practices.

Section II

Controls and Critical Limits for Biological, Chemical, and Physical Hazards

When all significant biological, chemical, and physical hazards are identified along with their points of occurrence, the next task is to identify measures to prevent the hazards from compromising the safety of the finished product.

Preventive measures or controls can be defined as physical, chemical, or other factors that can be used to remove or limit an identified hazard. When considering preventive measures or controls, a limit must be established—this is the criterion that must be met to ensure safety. For example, proper heat treatment will control some pathogenic bacteria, and it is thus crucial to know what time/temperature combinations constitute proper heat treatment for various products; these time/temperature combinations are the critical limits. Another example of a preventive measure for a biological hazard is the chlorination of poultry chiller water to prevent cross contamination of carcasses with *Salmonella*.

With identified physical hazards, the most common preventive measures may be visual examinations of product or the use of a metal detector. Chemical hazards associated with raw materials may be controlled through detailed product specifications, letters of guarantee, or purchase specifications.

Tables 4, 5, and 6 identify preventive measures that may be considered by the HACCP team. Table 7 gives some examples of regulatory limits.

TABLE 4.—EXAMPLES OF PREVENTIVE MEASURES FOR BIOLOGICAL HAZARDS

Pathogen	Preventive measure or control
Bacillus cereus	Proper holding and cooling temperatures of foods; thermal processing of shelf-stable canned food.
Campylobacter jejuni	Proper pasteurization or cooking; avoiding cross-contamination of utensils, equipment; freezing; atmospheric packaging.
Clostridium botulinum	Thermal processing of shelf-stable canned food; addition of nitrite and salt to cured processed meats; refrigeration of perishable vacuum packaged meats; acidification below pH 4.6; reduction of moisture below water activity of 0.93.
Clostridium perfringens	Proper holding and cooling temperatures of foods; proper cooking times and temperatures; adequate cooking and avoidance of cross-contamination by unsanitary equipment or infected food handlers.
Listeria monocytogenes	Proper heat treatments; rigid environmental sanitation program; separation of raw and ready-to-eat production areas and product.
Salmonella spp	Proper heat treatment; separation of raw and cooked product; proper employee hygiene; fermentation controls; decreased water activity; withdrawing feed from animals before slaughter; avoiding exterior of hide from contacting carcass during skinning; antimicrobial rinses; scalding procedures; disinfecting knives.
Staphylococcus aureus	Employee hygiene; proper fermentation and pH control; proper heat treatment and post-process product handling practices; reduced water activity.
Yersinia enterocolitica	Proper refrigeration; heat treatments; control of salt and acidity; prevention of cross-contamination.

TABLE 5.—EXAMPLES OF PREVENTIVE MEASURES FOR CHEMICAL HAZARDS

Hazard	Preventive measure
Naturally-Occurring Substances	Supplier warranty or guarantee; verification program to test each supplier's compliance with the warranty or guarantee.
Added Hazardous Chemicals	Detailed specifications for each raw material and ingredient; warranty or letter of guarantee from the supplier; visiting suppliers; requirement that supplier operates with a HACCP plan; testing program to verify that carcasses do not have residues.
In-Process Chemicals	Identify and list all direct and indirect food additives and color additives; check that each chemical is approved; check that each chemical is properly used; record the use of any restricted ingredients.

TABLE 6.—EXAMPLES OF PREVENTIVE MEASURES FOR PHYSICAL HAZARDS

Hazard	Preventive measure
Foreign objects in raw materials	Supplier's HACCP plan; use of specifications, letters of guarantee; vendor inspections and certification; in-line magnets; screens, traps, and filters; in-house inspections of raw materials.
Foreign objects in packaging materials, cleaning compounds, etc	Supplier's HACCP plan; use of specifications, letters of guarantee; vendor inspections and certification; in-house inspections of materials.
Foreign objects introduced by processing operations or employee practices.	In-line metal detectors; visual product examinations; proper maintenance of equipment; frequent equipment inspections.

TABLE 7.—SOME EXAMPLES OF REGULATORY LIMITS

Hazard	Regulatory limit	Regulatory citation
biological: Microbial growth due to temperature abuse-Poultry Chilling.	All poultry must be chilled immediately after processing to a temperature of 40 °F or less.	§ 381.66
chemical: Excess chemicals contact product	Chemicals used are approved for the intended use and at appropriate amounts.	§ 318.7
chemical: Chemical hazard from packaging materials	Edible products must be packaged in container that will not adulterate product or be injurious to health. Packaging materials must be covered by a letter of guaranty.	§ 317.24
biological: Trichinae in pork	Products containing pork muscle tissue must be effectively heated, refrigerated, or cured to destroy any possible live trichinae.	§ 318.10
biological: Pathogens in ready to eat products	For destruction of pathogens that may survive a dry heat process. One of the time/temperature combinations for *cooked* beef, *roast* beef, and *cooked* corned beef; e.g., 143 °F\61.7 °C minimum temperature at minimum time of 6 minutes.	§ 318.17
physical: Extraneous material found on post chill examination of poultry carcasses.	Sampled carcasses observed for conformance with post chill criteria, including unidentified foreign material.	§ 381.76

Section III

Table 8.—Red Meat (Beef) Slaughter Hazards and Controls Use of Information

This section contains examples of common process steps in beef slaughter. With each processing step, shown in the first column, you will find an ''X'' in the next three columns to tell you if there is a Biological hazard in column 2, a Chemical hazard in column 3, or a Physical hazard in column 4. Column 5 describes the hazard(s), and the last column lists some relevant controls or preventive measures. This table should be used in conjunction with the process flow diagram developed by your HACCP team for your plant's beef slaughter process.

TABLE 8.—RED MEAT SLAUGHTER: BEEF

Red meat slaughter-beef: examples of processing steps	B	C	P	Description of biological, chemical, or physical hazards for the process steps	Controls or preventive measures
Receiving & Holding		X		—Residues present in edible tissues above tolerances.	—Residue certification presented for live animal(s).
Skinning	X			—Micro contamination of carcass surface due to contaminated outside hide surface—contamination of carcass from floor—cross-contamination.	—Skinning procedures are accomplished without hair or visible fecal contamination of the carcass.—Careful employee practices.—Udder and puzzle removal are accomplished without contamination of edible product.
Evisceration	X			—cross-contamination from broken viscera.	—Esophagus is tied to prevent escape of stomach contents—Bung is dropped with sanitized knife and bagged to prevent escape of feces—Viscera are removed intact.
Final Wash	X			—growth of pathogens through insufficient wash.	—Final wash: Temperature: 90–100°F Pressure: 345–2070 kpa (50–300 psi)—Steam Pasteurization: Temperature: 195°F or greater at surface Dwell time: 5–15 seconds in cabinet.
Chilling	X			—growth of pathogens	—Surface temperature ≤40°F as soon as possible—Carcasses spaced a minimum of 1 inch apart.
Receiving-Packaging Materials and Non Beef Supplies.		X		—contamination from deletious chemicals present in the packaging materials.	Letters of guarantee on file for all packaging materials/non-poultry supplies used by the establishment.
Storage-Non Beef Supplies			X	—contamination of stored packing materials/supplies from foreign material.	Examine to ensure no visible foreign material on/in non-poultry supplies or packaging materials.

Section IV

Table 9.—Poultry Slaughter Hazards and Controls

Use of Information

This section contains examples of common process steps in poultry slaughter. With each processing step, shown in the first column, you will find an ''X'' in the next three columns to tell you if there is a Biological hazard in column 2, a Chemical hazard in column 3, or a Physical hazard in column 4. Column 5 describes the hazard(s), and the last column lists some relevant controls or preventive measures. This table should be used in conjunction with the process flow diagram developed by your HACCP team for your plant's poultry slaughter process.

TABLE 9.—POULTRY SLAUGHTER

Poultry slaughter: examples of processing steps	B	C	P	Description of biological, chemical, or physical hazards for the process steps	Controls or preventive measures
Scalding	X			—contamination from scalding medium	—Fresh water input to achieve a minimum of 1 quart per bird —Temperature of the scald water maintained at appropriate levels (e.g., ≥126°F) —Maintain counterflow scalding unit function —Post scald wash has sufficient pressure and volume to cover carcass with fresh (potable) water spray —Overflow volumes are at required amounts
Offline Procedures	X			—cross contamination from intestinal contents/exudate.	Follow approved offline plant procedures for handling airsacculitis salvage and reprocessing for contamination (e.g., an airsac salvage program that transfers the carcasses to another station where the thigh, drumstick, wing tip, and first wing section are salvaged and washed with chlorinated water).
Final Wash	X			—growth of pathogens	—A final water wash with appropriate levels of chlorinated water (e.g. 20–50 ppm residual chlorine in the water). —Sufficient water volume and pressure for equipment operation and sufficient dwell time in the final washer to remove visible contamination on internal and external surfaces of the carcass.
Chilling-Carcass	X			—growth of pathogens	Deep breast muscle temperature of carcass is ≤ 40°F within the specified time from slaughter for the class of poultry. —Maintain an adequate chlorine level in the overflow water of in-line immersion chillers (e.g., 20–50 ppm residual chlorine in the incoming water). —Maintain proper water flow rates (input/overflow) for continuous chillers per USDA requirements (not less than ½ gallon of fresh water per frying chicken with continuous overflow).
			X	—contamination from foreign material	Product entering (prechill) and exiting (postchill) the chiller system meets the criteria for defects per USDA requirements (e.g. the limits are not exceed for the number and size of extraneous materials found during the postchill examination-9 CFR § 381.76).
Chilling-Giblet/Neck	X			—growth of pathogens	—Temperature and fresh water input sufficient to meet USDA requirements for giblets and necks. —Chlorination of giblet chiller water at appropriate levels for giblets and necks [e.g., giblets must be chilled to 40°F within 2 hours from removal from other viscera/fresh water intake not less than 1 gallon per 40 frying chickens processed-9 CFR § 381.66 (c)(5)].
			X	—contamination from foreign material	—Visually free of hazardous foreign material. —Defects on poultry giblet and necks meet USDA requirements (e.g., each carcass must be observed for conformance against pre and post chill criteria, including unidentified foreign materials-MPI Regulations 381.76).

TABLE 9.—POULTRY SLAUGHTER—Continued

Poultry slaughter: examples of processing steps	B	C	P	Description of biological, chemical, or physical hazards for the process steps	Controls or preventive measures
Cut-Up/Boning/Packaging/ Labeling	X			—growth of pathogens	Temperature of product does not exceed 55°F during further or second processing. —Movement of product through these areas and into the cooler is timely and efficient. —A mid-shift cleanup of the area(s) is performed if the room temperature is not maintained at or below 50°F. —Packaging/labeling materials that come into direct contact with product are intact.
Receiving-Packaging Materials and Non Poultry Supplies.		X		—contamination from deleterious chemicals present in the packaging materials.	Letters of guarantee are on file for all packaging materials/non-poultry supplies used by the establishment.
Storage-Non Poultry Supplies			X	—contamination of stored packing materials/supplies from foreign material.	Examine to ensure no visible foreign material on/in non- poultry supplies or packaging materials.

Section V

Table 10.—Red Meat (Swine) Slaughter Hazards and Controls

Use of Information

This section contains examples of common process steps in swine slaughter. With each processing step, shown in the first column, you will find an ''X'' in the next three columns to tell you if there is a Biological hazard in column 2, a Chemical hazard in column 3, or a Physical hazard in column 4. Column 5 describes the hazard(s), and the last column lists some relevant controls or preventive measures. This table should be used in conjunction with the process flow diagram developed by your HACCP team for your plant's swine slaughter process.

TABLE 10.—RED MEAT SLAUGHTER: SWINE

Red meat slaughter-swine: Examples of processing steps	B	C	P	Description of biological, chemical, or physical hazards for the process steps	Controls or preventive measures
Scalding	X		X	—contamination from scalding medium ...	Plant time/temperature limits for scalding (e.g., although it may vary with facilities, a temperature of 138 to 140°F is usually satisfactory). —Carcasses should remain in scalding tanks long enough to loosen hair (excessive time or temperature results in carcass cooking).
		X	....	—contamination with chemicals.	—USDA-FDA approved chemical concentration not to exceed manufacturer's recommendations.
Dehairing	X	....	....	—contamination and growth of microorganisms due to breaking of the skin from overexposure to the dehairer.	—Time/temperature determined by plant-specific testing results to remove visible hair to an acceptable level without breaking skin.
Evisceration	X	....	....	—cross contamination from equipment/ utensils. —contamination from stomach, intestines, and/or bladder contents. —contamination from employee handling	—Remove all viscera intact. —Contaminated equipment will be clean and sanitized before being used again. —Training program for all employees, to include personal hygiene, product handling procedures, and sanitary dressing procedures.
Trimming	X	....	....	Stick wound has not been removed.	Remove all visible stick-wound related defects.
Chilling	X	....	....	—growth of pathogens	—Cool surface temperature to 40° as soon as possible.
Receiving-Packaging Materials and Non Swine Supplies.	....	X	....	—contamination from deleterious chemicals present in the packaging materials.	Letters of guarantee are on file for all packaging materials/non-poultry supplies used by the establishment.
Storage-Non Swine Supplies	....		X	—contamination of stored packing materials/supplies from foreign material.	Examine to ensure no visible foreign material on/in non-poultry supplies or packaging materials.

Section VI

Table 11.—Ingredient Hazards and Ingredient-Related Hazards

Use of Information

This section contains an alphabetical list of ingredients commonly used in making meat and poultry products. For each entry you will find the name of the ingredient in the first column, and an ''X'' in the next three columns to tell you if there is a Biological hazard in column 2, Chemical hazard in column 3, or Physical hazard in column 4. Column 5 describes the hazard(s), and the last column lists some relevant controls or preventive measures. This table should be used in conjunction with the list of ingredients developed by your HACCP team for the products produced by the process under consideration.

The HACCP team may find that a particular ingredient does not present the hazard identified in these tables. The presence or absence of a hazard can be influenced by the ingredient source and company. Also, *Ingredient Specifications,* provided by the supplier to the establishment, may give details on the material/ingredient being sold, including statements that the materials/ ingredients are food grade and are free of harmful components. For example, the ingredient specifications for dried legumes might state that there will be fewer than 5 small rocks or stones per 10 pound bag and that no harmful pesticides were used in the growing process.

TABLE 11.—INGREDIENT HAZARDS

Examples of ingredient	B	C	P	Description of biological, chemical, or physical hazard for the ingredient	Controls or preventive measures
Acidifiers	....	X	....	—toxicological effects if limits are exceeded.	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/provider ingredient specifications.
Anticoagulants	....	X	....	—toxicological effect if limits are exceeded.	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/provider ingredient specifications.
Antifoaming agents	....	X	....	—toxicological effect if limits are exceeded.	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/ provider ingredient specifications.
Antioxidants	....	X	....	—toxicological effect if limits are exceeded.	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/provider ingredient specifications.
Batter/Breading	X	....	X	—growth of pathogens due to improper storage and handling. —foreign material	—Temperature controls for use —Ingredient specification sheet identifying the required parameters the ingredient must meet. —Where applicable, ingredients must be pathogen-free.
Beef (fresh, frozen)	X	....	....	—growth of pathogens due to improper storage and handling.	—Product temperature must be 40 degrees F or less at receiving. —Product must meet establishment purchase specifications. —Product must be produced under a HACCP plan.
Binders/Extenders	....	X	X	—foreign material	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/ provider ingredient specifications.
Bleaching agents	....	X	....	—toxicological effect if limits exceeded	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/ provider ingredient specifications.
Blood	X	....	....	—growth of pathogens from improper handling and storage.	—Ingredient specification sheet identifying the required parameters the ingredient must meet. —Where applicable, ingredients must be pathogen-free. —Meet appropriate temp.
Boneless beef	X	....	X	—growth of pathogens due to improper handling and storage. —foreign particle contamination, e.g., metal fragments or bone.	—Product temperature must be 40 degrees F or less at receiving. —Product must meet establishment purchase specifications. —Product must be produced under a HACCP plan. —Visual examination of product for foreign materials.

TABLE 11.—INGREDIENT HAZARDS—Continued

Examples of ingredient	B	C	P	Description of biological, chemical, or physical hazard for the ingredient	Controls or preventive measures
Cooked beef	X	....	X	—growth of pathogens due to improper handling and storage. —foreign particle contamination, e.g., metal fragments or bone particles in boneless beef.	—Receiving temperature of product must be frozen or refrigerated at 40 degrees F or below. —Product must be received from an approved supplier who produces the product under a HACCP plan. —Visual examination of product for foreign materials upon receipt.
Cooked poultry	X	....	X	—growth of pathogens due to improper handling and storage. —foreign particle contamination, e.g., bone particles in boneless poultry.	—Receiving temperature of product must be frozen or refrigerated at 40 degrees F or below. —Product must be received from an approved supplier who produces the product under a HACCP plan. —Product must be organoleptically acceptable at receipt.
Cooked pork	X	....	X	—growth of pathogens due to improper handling and storage. —foreign particle contamination, e.g., bone particles in boneless pork.	—Receiving temperature of product must be frozen or refrigerated at 40 degrees F or below. —Product must be received from an approved supplier who produces the product under a HACCP plan. —Product must be organoleptically acceptable at receipt.
Coloring agents (natural)	....	X	....	—Toxicological effect if limits exceeded	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/provider ingredient specifications.
Coloring agents (artificial)	....	X	....	—Toxicological effect if limits exceeded	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/provider ingredient specifications.
Curing agents	....	X	....	—Toxico logical effect if limits exceeded	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/provider ingredient specifications.
Curing accelerators	....	X	....	—-toxicological effect if limits are exceeded.	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/provider ingredient specifications.
Dairy products	X	....	X	—growth of pathogens due to improper handling and storage. —foreign material	—Temperature control. —Ingredient specification sheet identifying the required parameters the ingredient must meet. —Where applicable, ingredients must be pathogen-free.
Eggs or egg products	X	....	X	—growth of pathogens due to improper handling and storage. —foreign particle contamination, e.g., shell particles in broken eggs.	—Temperature control. —Ingredient specification sheet identifying the required parameters the ingredient must meet. —Where applicable, ingredients must be pathogen-free.
Emulsifying agents	....	X	....	—toxicological effects if limits exceeded	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/provider ingredient specifications.
Flavoring agents	....	X	....	—toxicological effects if limits exceeded	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/provider ingredient specifications.
Fruits	....	X	X	—contamination from agricultural chemicals. —foreign material	—Ingredient specification sheet identifying the required parameters the ingredient must meet.
Honey	X	....	X	—contamination from inherent microorganisms. —foreign particle contamination, e.g., dirt, insect parts.	—Ingredient specification sheet identifying the required parameters the ingredient must meet.
Legumes (dry)	....	....	X	—foreign particle contamination, e.g., rocks.	—Ingredient specification sheet identifying the required parameters the ingredient must meet.

TABLE 11.—INGREDIENT HAZARDS—Continued

Examples of ingredient	B	C	P	Description of biological, chemical, or physical hazard for the ingredient	Controls or preventive measures
Mechanically deboned product	X	....	X	—growth of pathogens due to improper handling and storage. —foreign particle contamination, e.g., bone particles.	—Product temperature must be 40 degrees F or less at receiving. —Product must meet establishment purchase specifications. —Product must be produced under a HACCP plan.
Mold inhibitors	....	X	....	—toxicological effect if improper amounts used.	—Ingredient specification sheet identifying the required parameters the ingredient must meet.
Mushrooms	X	X	X	—contamination from inherent microorganisms. —contamination from agricultural chemicals. —foreign material	—Ingredient specification sheet identifying the required parameters the ingredient must meet. —Where applicable, ingredients must be pathogen-free.
Nuts	X	X	X	—contamination from inherent microorganisms. —contamination from agricultural chemicals. —foreign particle contamination, e.g., broken shells.	—Ingredient specification sheet identifying the required parameters the ingredient must meet.
Packaging materials	....	....	X	—toxicological effects	—Use only FDA approved packaging materials. — Each lot of packaging material must be accompanied by a Letter of Guarantee in which the manufacturer attests to compliance with FDA requirements.
Phosphates	....	X	....	—toxicological effect if limits are exceeded.	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/provider ingredient specifications.
Poultry (fresh, frozen)	X	....	....	—growth of pathogens due to improper handling and storage.	—Product temperature must be 40 degrees F or less at receiving. —Product must meet establishment purchase specifications. —Product must be produced under a HACCP plan.
Pork (fresh, frozen)	X	....	....	—growth of pathogens due to improper handling and storage.	—Product temperature must be 40 degrees F or less at receiving. —Product must meet establishment purchase specifications. —Product must be produced under a HACCP plan.
Proteolytic enzymes—Aspergillus oryzae, Aspergillus, Flavusoryzae group, Bromelin, Ficin, Papain.	....	....	....	—toxicological effects if limits exceeded	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/provider ingredient specifications.
Partially defatted products	X	....	X	—growth of pathogens due to improper handling and storage. —foreign particle contamination, e.g., metal, plastic.	—Product temperature must be 40 degrees F or less at receiving. —Product must meet establishment purchase specifications. —Product must be produced under a HACCP plan.
Seafood (fresh, frozen)	X	X	....	—growth of pathogens due to improper handling and storage. —environmental contamination	—Product temperature must be 40 degrees F or less at receiving. —Product must meet establishment purchase specifications. —Product must be produced under a HACCP plan.
Spices/herbs—Sterilized, Unsterilized	X	....	....	—contamination from microorganisms inherent to the ingredient. —contamination from agricultural chemicals. —foreign material	—Ingredient specification sheet identifying the required parameters the ingredient must meet.
Sweeteners—Saccharin, Citric acid, Malic acid, Monoisopropyl citrate, Phosphoric acid, Monoglyceride citrate.	....	....	....	—toxicological effects if limits exceeded	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/provider ingredient specifications.

TABLE 11.—INGREDIENT HAZARDS—Continued

Examples of ingredient	B	C	P	Description of biological, chemical, or physical hazard for the ingredient	Controls or preventive measures
Tenderizing agents	....	X	....	—toxicological effects if limits exceeded	—Ingredients purchased under a Letter of Guarantee. —Ingredients purchased based on producer/provider ingredient specifications.
Variety meats	X	....	....	—growth of pathogens due to improper handling, storage, or cleaning.	—Product temperature must be 40 degrees F or less at receiving. —Product must meet establishment purchase specifications. —Product must be produced under a HACCP plan.
Vegetables	X	X	X	—growth of pathogens due to improper handling and storage. —contamination from agricultural chemicals. —foreign material	—Ingredient specification sheet identifying the required parameters the ingredient must meet.

Section VII

Table 12.—Processing Hazards and Controls

Use of Information

This section contains a list of processing hazards and controls commonly used in making meat and poultry products. They are listed in alphabetical order. For each processing step, shown in the 1st column, you will find an ''X'' in the next three columns to tell you if there is a Biological hazard in column 2, Chemical hazard in column 3, or Physical hazard in column 4. Column 5 describes the hazard(s), and the last column lists some relevant controls or preventive measures. This table should be used in conjunction with the process flow diagram developed by your HACCP team for the products produced during the process under consideration.

TABLE 12.—PROCESSING STEP HAZARDS

Processing steps	B	C	P	Description of biological, chemical, or physical hazards for the process steps	Controls or preventive measures
Acidifying (also see Pickling, Brining)	X	....	....	—survival of pathogens due to final pH>4.6.	—Shelf-stable non-heat treated acidified product must obtain a pH of 4.6 or lower.
Aging (Meats)	X	....	....	—growth/survival of pathogens from inappropriate storage temperatures and humidity (inadequate product water activity (a_w)). —growth of pathogens due to rise in the pH due to development of surface molds.	—The temperature of the aging room will not exceed 40 degrees Fahrenheit. —Product temperature does not exceed 40 degrees Fahrenheit throughout the aging process. —The aging process will not exceed seven days.
Boning	X	....	....	—contamination by pathogens in product accumulations (e.g., cutting boards, conveyor belts, utensils and other equipment). —cross-contamination of product by equipment/utensils contaminated with pathogens when cutting through a non-apparent lesion (e.g., abscesses). —contamination from bones, cartilage/extraneous material.	—Careful employee practices to make sure that there is no contamination of the product. —Equipment and utensils are washed and sanitized immediately when contaminated and each time the employee leaves the working station. —All hot water sanitizers are maintained at 180 degrees Fahrenheit. —Processing room temperature is maintained at 50 degrees Fahrenheit, or a midshift cleanup is performed within five hours after operations begin. —A boneless beef re-inspection procedure will be established using specifications outlined by FSIS.
Cooling	X	....	....	—growth of pathogens due to improper temperatures. —germination of spore-forming pathogens due to slow chilling (e.g., *C. perfringens*).	Cooked product will be cooled according to established procedures.
Cooking	X	....	....	—survival of pathogens due to improper procedures.	—Time/Temperature combinations are adequate to destroy the pathogens of concern.

TABLE 12.—PROCESSING STEP HAZARDS—Continued

Processing steps	B	C	P	Description of biological, chemical, or physical hazards for the process steps	Controls or preventive measures
Drying (Meat)	X	....	....	—bacterial growth due to inadequate control over time, temperature and humidity.	—A water activity will be specified that in conjunction with other barriers will inhibit growth of pathogenic microorganisms (e.g., for shelf stable sausage A_w of 0.91 and a pH of 4.6).
Filling	X	....	....	—recontamination by pathogens in product accumulations. —growth of pathogens due to temperature abuse.	—Product will be protected from contamination during the filling process, and product temperature/ time will be maintained at or below the maximum determined to inhibit growth of pathogenic microorganisms.
	....	X	....	—contamination from lubricants	—No lubricants or other chemical contaminants will be allowed in or on the product.
Formulation	X	....	....	—contamination by employee handling —incorrect formulation —contamination through damaged packages.	—Careful employee practices used at all times to make sure that there is no contamination of product. —Ingredient packages will be clean and intact. —Ingredients will be added to product according to requirements outlined 9CR § 318.7.
	....	X	....	—excessive addition of restricted ingredients/ additives could be toxic to the consumer.	—Restricted ingredients will be added to product according to requirements outlined in the 9CFR § 317.8.
Freezing (Meats)	X	....	....	—survival of parasites due to improper time/temperature application. —growth of pathogens due to temperature abuse.	—Rapid cooling and freezing.
Grinding	X	....	....	—contamination by employee handling —recontamination by pathogens in product accumulations.	—Careful employee practices to make sure that there is no contamination of product. —Product will not be allowed to accumulate at the end of the grinder. —The temperature of the grinding room will be maintained at 50 degrees Fahrenheit.
Grinding	....	X	....	—contamination from lubricants	—Food grade lubricants will be used on areas of the machinery where a potential for product contamination exists.
	....	....	X	—contamination from extraneous material	—All boneless product will be re-inspected before being loaded into the grinder.
Handling and Inspecting of Empty Containers and Packaging Materials.	X	X	X	—recontamination through damaged or soiled containers/packaging material.	—Packaging materials and empty containers will be protected from contamination during their storage and handling. —No materials or containers that appear to be contaminated with hazardous foreign material will be used.
Mechanical Separating	X	....	....	—growth of pathogens	—Product holding and cooling requirements outlined in 9CFR 318.18 will be followed.
			X	—contamination from bone, cartilage fragments. —contamination from extraneous material	—The finished product will meet the standards outlined in 9CFR 319.5 for bone particles and calcium.
Packaging (also see Modified Atmosphere Packaging, Vacuum Packaging Seaming, Sealing).	X	X	X	—contamination from packaging material —contamination through damaged containers.	—Closure and/or machine specifications sufficient to ensure adequate barrier formation.
	....	....	X		—No detectable foreign material will be allowed in or on the product or immediate product containers.
Peeling	X	....	....	—contamination by pathogens in product accumulations. —contamination from employee handling	—Careful employee practices to make sure that there is no contamination of product. —Product will not be allowed to accumulate in/on peeling equipment.
	....	....	X	—contamination from harmful extraneous material.	—Peeling equipment will be maintained in a proper operating condition. No foreign material in the finished product.

TABLE 12.—PROCESSING STEP HAZARDS—Continued

Processing steps	B	C	P	Description of biological, chemical, or physical hazards for the process steps	Controls or preventive measures
Receiving	X	....	....	—contamination through damaged containers. —growth of pathogens due to inappropriate storage conditions (temperature, humidity). —growth of pathogens due to temperature abuse. —contamination from receiving equipment (pumps, hoses).	—Product must be received in sound containers and at temperatures appropriate for the type of product.
	....	X	....	—cross-contamination from non-food chemicals.	—Product must be received in sound containers and be accompanied by a letter of guarantee from the supplier if such letter is not on file.
	....	X	....	—contamination from hazardous extraneous material (wood, nails from pallets, plastic pieces).	—Product must be received in sound containers and be accompanied by a letter of guarantee from the supplier if such letter is not on file.
Retorting	X	....	....	—inadequate application of scheduled process.	—A thermal process specific to the product, container type and size, and retorting system must be in use. The initial product temperature and any critical factors specified for the thermal process must also be controlled. Specified retort come up procedures will be followed.
Reworking	X	....	....	—contamination by employee handling ... —contamination by pathogens in product accumulations.	—Careful employee practices to make sure that there is no contamination of product. —Room temperature of storage coolers will not exceed 40 degrees Fahrenheit.
	....	....	X	—contamination foreign material	—Careful employee practices to make sure that there is no contamination of product.
Shipping	X	....	....	—growth due to improper temperatures	—Product will not be shipped unless it is 40 degrees Fahrenheit or less. —Product will not be loaded into transport vehicles if the trailer temperature exceeds 40 degrees Fahrenheit.
	....	....	X	—contamination from hazardous extraneous material through damaged packages.	—All product packages will be intact before shipping. —All transport vehicles will be cleaned after each use and before loading of product.
Thawing	X	....	....	—growth of pathogens due to improper temperatures.	—Thawing Room temperature will not exceed 50 degrees Fahrenheit.

Section VIII

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Appendix E—FSIS Sample Collection Guidelines and Procedure for Isolation and Identification of Salmonella from Raw Meat and Poultry Products

Introduction

This sampling protocol has been prepared to support the Pathogen Reduction/HACCP Regulation. FSIS will be conducting a *Salmonella* testing program in support of this regulation. The regulation does not require establishments to conduct their own testing for *Salmonella*. However, for those who choose to conduct their own *Salmonella* testing program, the protocol outlined in this document provides detailed instruction for sample collection and analysis that are the same as those used in the FSIS *Salmonella* testing program for raw meat and poultry products.

This protocol incorporates the use of a non-destructive sampling technique for sample collection of raw beef and swine carcasses. These techniques have been evaluated by the Agricultural Research Service and have been designed to give comparable results to the FSIS Nationwide Microbiological Baseline Data Collection Programs' excised tissue samples. We are continuing to improve the sponging techniques and welcome comments. This technique will be closely monitored during the first year of prevalence phase *Salmonella* testing. Carcass sampling for broiler and turkey carcasses remain the nondestructive whole bird rinse which was used in the Baseline Programs. Ground product sampling involves collecting approximately ½ pound of the product.

The analytical methods section of this protocol details the cultural procedures currently in use by FSIS/USDA for the examination of raw meat and poultry products for *Salmonella*. Any screening method under consideration for *Salmonella* testing must meet or exceed the following performance characteristics: sensitivity = ≥97%, specificity ≥96%, false-negative rate = 3%, false-positive rate ≤4%.

Guidelines for Sample Collectors/Microbiologists

Pre-Sampling Preparation

Prior to collecting samples, the individual designated for sample collection should compile a written establishment-specific sample collection protocol for microbiological analysis. This protocol should include a check list for tasks to be performed prior to sample collection, materials needed for sample collection, random selection procedures, where the samples will be analyzed (on-site versus off-site), and other information that will aid the sample collector. Sampling supplies, such as sterile gloves, sterile sampling solutions, hand soap, sanitizing solution, etc., as well as specific materials needed for sampling different carcass types (i.e., specimen sponges in bags, if sampling cattle or swine carcasses), will need to be assembled.

For cattle and hog carcass sampling, a template will be needed to mark off the area to sample (Figure 1). The template can be made of metal or aluminum foil, brown paper, etc. From a sheet larger than the area to be sampled, cut out a 10 cm (3.94 inches) x 10 cm square for sampling cattle or a 6 cm x 10 cm rectangle for swine carcass sampling. If a reusable metal template is used, it will need to be sanitized with an approved sanitizing solution (e.g. hypochlorite (bleach) solution or alcohol). However, the template needs to be dry before placing it on the carcass. Aluminum foil or paper templates can be used once and discarded. The foil for the template should be stored in a manner to prevent contamination. Since the area enclosed by the template will be sampled, take care not to touch this area with anything other than the sampling sponge. Using dirty or contaminated material may lead to erroneous results. If an autoclave is available, paper or aluminum foil templates can be wrapped in autoclavable paper and sterilized.

The sterile sampling solution, Buffered Peptone Water (BPW), can be stored at room temperature. However, at least one day prior to sample collection, check solutions for absence of cloudiness and/or turbidity and place the number of containers of sampling solution (BPW) that will be needed for the next day's sampling in the refrigerator. DO NOT use solutions that are cloudy, turbid, or contain particulate matter.

To obtain the most accurate results, samples should be analyzed as soon after collection as possible. However, if samples must be transported to an off-site laboratory, the samples need to be

maintained at refrigeration temperatures until transport, then shipped refrigerated via an overnight delivery service to the laboratory performing the analysis. Samples analyzed off-site must be picked up by the overnight courier the SAME calendar day the sample is collected. The sample must arrive at the laboratory no later than the day after the sample is collected. Samples shipped to an outside laboratory must be analyzed no later than the day after collection. The following section gives information on shipping containers and transporting samples to off-site facilities.

Shipping Containers and Coolant Packs

It is important that samples fit easily into the shipping so that the sample bags do not break.

Correct use of the refrigerant gel-ice packs and proper packing of the shipping container are necessary so that samples arrive at the laboratory at an acceptable temperature. Frozen samples or samples which are too warm are not considered valid and must not be analyzed. Some bacteria may be damaged by temperatures that are too cold. Temperatures that are too warm can allow bacteria to reproduce. Maintaining samples at improper temperatures may cause inaccurate sample results.

The sample should be kept refrigerated, NOT FROZEN, in the shipping container prior to pickup by the courier. The shipping container, itself, should not be used as a refrigerator. However, multiple samples (if needed) for that day may be stored in the open shipping container in the cooler or refrigerator.

Random Selection of Carcasses or Ground Product for Sampling

Samples are to be taken randomly. There are different methods of selecting the specific carcass for sampling that could be used but all require the use of random numbers. Methods could include: using random number tables, drawing cards, using calculator- or computer-generated random numbers, etc. When selecting the random numbers, use the method(s) currently in use at the establishment for other sampling programs, if other programs are currently underway.

The carcass or ground product for sampling must be selected at random from all eligible carcasses. If multiple lines exist, randomly select the line for sample collection for that interval. Repeat the random selection process for the next sampling interval. Each line should have an equal chance of being selected at each sampling interval.

Cattle Carcass Selection

The half-carcasses eligible for sampling should be selected from those in the cooler 12 or more hours after slaughter. Both the ''leading'' and ''trailing'' sides of a carcass should have an equal chance of being selected. NOTE: If more than one shift is operating at the plant, the sample can be taken on any shift, provided the following requirements are met:

Selection of TIME: Determine the times that carcasses chilled for 12 or more hours will be on hand. Then randomly select a time for collecting samples. If samples are shipped off-site, then take into account that the delivery service may have limitations on pickup times.

Selection of COOLER SITE: Select a safe and accessible site in the cooler for random selection of the half-carcass. This site may be located at the transfer chain, grading chain, or a rail that contains carcasses that have been chilled 12 hours or more.

Selection of HALF–CARCASS: At the random time selected, identify a half-carcass (selected by your random number method) from the predetermined point along the chain (selected cooler site) and then count back five (5) half-carcasses and select the next half-carcass (carcass) for sampling. The reason for counting back five half-carcasses is to avoid any possible bias during selection.

Swine Carcass Selection

The carcasses eligible for sampling should be selected from those in the cooler 12 or more hours after slaughter. Every carcass should have an equal chance of being selected.

Note: If more than one shift is operating at the plant, the sample can be taken on any shift, provided the following requirements are met:

Selection of TIME: Determine the times that carcasses chilled for 12 or more hours will be on hand. Then randomly select a time for collecting samples. If samples are shipped off-site, then take into account that the delivery service may have limitations on pickup times.

Selection of COOLER SITE: Select a safe and accessible site in the cooler for random selection of the carcass. This site may be located at the transfer chain, or a rail that contains carcasses that have been chilled 12 hours or more. If there are multiple sites of the same kind, select one at random.

Selection of CARCASS: At the random time selected, identify a carcass (selected by your random number method) from the predetermined point along the chain and then count back five (5) carcasses and select the next carcass for sampling. The reason for counting back five carcasses is to avoid any possible bias during selection.

Poultry Carcass Selection

The poultry carcasses will be selected at random after chilling, at the end of the drip line or last readily accessible point prior to packing/cut-up. A WHOLE carcass is required, that is, one that has not been trimmed.

Note: If more than one shift is operating at the plant, the sample can be taken on any shift, provided the following requirements are met:

Selection of TIME: Determine the times that chilled carcasses will be on hand, then randomly select a time for collecting samples. If samples are shipped off-site, then take into account that the delivery service may have limitations on pickup times.

Selection of CHILLER: If more than one chiller system is in operation at the time of sample collection, the chill tank from which the sample is selected must be randomly selected.

Selection of POULTRY CARCASS: At the random time, identify a carcass (selected by your random number method) from the predetermined point, and then count back five (5) carcasses and select the next carcass for sampling. Exception: If the fifth carcass is not a WHOLE (untrimmed) bird, count back an additional five carcasses for sample selection. Remember: Each carcass must have an equal chance of being selected. The reason for counting back five carcasses is to avoid any possible bias during selection.

Raw Ground Product Selection (Beef, Pork, Chicken, Turkey)

Raw ground product samples will be randomly selected and collected after the grinding process and, if possible before any addition of spices or seasonings, but prior to final packaging.

Note: If more than one shift is operating at the plant, the sample can be taken on any shift, provided the following requirements are met:

Selection of TIME: Determine the times that raw ground product will be produced, then randomly select a time for collecting samples. Take into account that the overnight delivery service may have limitations on pickup times, for determining sample collection time.

Selection of GRINDER: If more than one grinder is in operation at the time of sample collection, the grinder from which the sample is selected must be randomly selected.

Aseptic Techniques/Sampling

Extraneous organisms from the environment, hands, clothing, sample containers, sampling devices, etc., may lead to erroneous analytical results. Stringent requirements for microbiological analysis are necessary, therefore, use of aseptic sampling techniques and clean sanitized equipment and supplies are of utmost importance. The following information gives general techniques for aseptic techniques that are routinely used during sample collection for microbiological analysis.

There should be an area designated for preparing samples, etc. A stainless steel, wheeled cart or table would be useful during sampling. A small tote or caddy could be could be easily transported to the location of sampling and used for carrying supplies, supporting sample bags when adding sterile solutions to sample bags, etc.

Sterile gloves should be used for collecting samples. The only items which may contact the external surface of the glove are the exposed sample being collected and/or the sterile sample utensil (specimen sponge). Keep in mind that the outside surfaces of the sample container are not sterile. Do not handle the inside surface of the sterile sample containers. Do not touch anything else. The following procedure for putting on sterile gloves can be followed when collecting samples:

(a) Peel open the package of sterile gloves from the top without contaminating (touching, breathing on, contacting, etc.) the exterior of the gloves.

(b) Remove a glove by grasping it from the wrist-side opening inner surface which is folded. Avoid any contact with the outer surface of the glove. Insert the washed and sanitized hand into the glove, taking care not to puncture the glove or touch the outside surface of the glove.

(c) Next, follow the same procedure for the hand you will use to physically handle the sample, using care not to contaminate the outer surface of the glove.

(d) If at any time you are concerned that a glove may be contaminated, discard it and begin again with Step (a) above.

Preparation for Sample Collection

Prior to collecting samples, review steps for sample collection, random selection procedure, etc.

At least one or more days prior to sample collection, check sampling solution (BPW) for cloudiness/turbidity and refrigerate if not cloudy or turbid. If shipping samples to off-site facility, place coolant packs in freezer then pre-chill open shipping in cooler/refrigerator.

On the day of sampling, gather all sample collection bags, sterile gloves, sanitizer, hand soap, sterile solutions for sampling, and specific materials listed under the *Materials* section of the sample collection section for the type of carcass to be sampled.

Label the sample bags before starting sampling procedure. Use permanent ink. If you are using paper labels, it is important that the label be applied to the bag at normal room temperature; it will not stick if applied in the cooler.

Outer clothing (frocks, gloves, head gear, etc.) worn in other areas of the plant should be removed before entering the sampling area or preparing to collect samples. Replace outer clothing removed earlier with clean garments (i.e. laboratory coat) that have not been directly exposed to areas of the plant outside of the sampling area.

Sanitize the sample work area surfaces by wiping with a clean disposable cloth or paper towel dipped in a freshly prepared 500 ppm sodium hypochlorite solution (0.05% sodium hypochlorite) or other approved sanitizer which provides an equivalent available chlorine concentration. The sample work area surfaces must be free of standing liquid before sample supplies and/or product containers are placed on them.

Before sampling, thoroughly wash and scrub hands to the mid-forearm. Use antibacterial hand soap. If available, this should include a sanitizer at 50 ppm equivalence available chlorine. Dry the hands using disposable paper towels.

Specific Sample Collection Procedures

Raw Ground Product

Materials

1. 2 sterile ziplock-type or stomacher bags or equivalent.
2. Sterile gloves.
3. Plastic cable-tie-wrap or thick rubber band for securing bag.

Collection

Ensure that all supplies are on hand and readily available. Use the predetermined random selection procedure to select sample. Samples of raw ground product will be collected after the grinding process, and, if possible, before the addition of any spices or seasonings, but prior to final packaging.

1. Put on sterile gloves.

2. Aseptically collect approximately ½ pound of ground product, if possible, before the addition of any spices or seasonings, but just prior to final packaging. (Sample will be about the size of an orange.) Use the sterile sampling bag, taking care not to contaminate the inside of the bag with your gloved hand.

3. Close the bag tightly by twisting the top and securing it with the plastic cable-tie-wrap or rubber band or securely closing the ziplock-type bag.

4. Place bagged sample inside a second bag and close the outer bag tightly.

5. (a) If samples are to be analyzed at an ON–SITE LABORATORY, begin sample preparation for analysis.

(b) If samples are to be analyzed at an OUTSIDE (OFF–SITE) LABORATORY, follow the procedure in the Sample Shipment section.

Cattle Surface Sample Collection Procedure

Materials

1. Sterile specimen sponge in sterile Whirl-Pak® bag or equivalent
2. 10 ml sterile Buffered Peptone Water (BPW)
3. Sterile ziplock-type or stomacher bag
4. Template for a 100 cm^2 sampling area
5. Sterile gloves
6. Wheeled ladder, sampling platform, or step ladder
7. Sanitizing solution
8. Small tote or caddy for carrying supplies

Collection

A sterile, moistened sampling sponge (which usually come pre-packaged in a sterile bag) will be used to sample all three sites on the swine carcass (ham, belly, and jowls—see Figure 3). It is important to swab the sampling areas in the order of least to most contaminated to avoid spreading any contamination on the carcass. Therefore, swab sampling areas in the sequence indicated in this protocol. Use predetermined random selection procedures for selecting carcass to be sampled. Remember: samples will be collected from carcasses in the cooler 12 hours or more after slaughter. Nondestructive surface sampling will be conducted as follows:

1. Ensure that all bags have been pre-labeled and all supplies are on hand, including the sampling template. (An assistant may be helpful during the sampling process.)

2. Position the wheeled ladder, sampling platform, or step ladder near the carcass so the rump sample area (Figure 2) is within easy reach from the ladder.

3. IF a reusable template is used, have the assistant immerse the sampling

template in a sanitizing solution for at least 1–2 minutes. Just prior to taking the first sample on the carcass, have the assistant put on a pair of gloves (taking care not to contaminate the outer surface of the glove with fingers) and retrieve the sampling template from the sanitizing solution. Shake excess solution from utensil, then protect the portion of the template that will contact the carcass from contamination.

4. Locate the flank, rump, and brisket sampling sites using illustrations and directions in Figure 2 (cattle carcass sampling locations).

5. To hydrate the sponge, open the sponge bag. Remove cap from sterile BPW bottle, being careful not to touch the bottle opening. Carefully pour the contents of the sterile BPW bottle (10 ml) into the sponge bag to moisten the sponge.

6. Close the top of the bag. Use hand pressure from the outside of the bag and carefully massage the sponge until it is FULLY HYDRATED (moistened).

7. With the bag still closed, carefully push the moistened sponge to the upper portion of the bag orienting one narrow end of the sponge up toward the opening of the bag. Do NOT open the bag or touch the sponge with your fingers.

8. Open the bag containing the sponge, being careful not to touch the inner surface of the bag with your fingers. The wire closure at the top of the bag should keep the bag open. Set bag aside.

9. Put on sterile gloves.

10. Carefully remove the moistened sponge from the bag with your sampling hand. Take care to avoid touching the surfaces of the sampling sponge.

11. With the other hand, retrieve the template by the outer edge taking care to avoid contaminating the inner edges of the sampling area of the template.

12. Locate the flank sampling area (Figure 2) and place template over this location.

13. Hold the template in place with one gloved hand. Take care not to contaminate the enclosed sampling area with your hands.

14. With the other hand, wipe the sponge over the entire enclosed area (10 cm×10 cm) for the sample for a total of approximately 10 times in the vertical and 10 times in the horizontal directions. The pressure for swabbing would be as if you were removing dried blood from the carcass. However, the pressure should not be too hard as to crumble or destroy the sponge. (Note: The template may need to be ''rolled'' from side to side during swabbing since the surface of the carcass is not flat. This ensures that the 100 cm^2 area is enclosed while swabbing.)

15. Repeat steps 13–15 for the brisket area, using the SAME side or surface of the sponge used to swab the flank sampling area.

16. After swabbing the brisket area, transfer the template to the same hand holding the sponge. Do not contaminate the inner edges of the sampling area of the template.

17. Climb the ladder or platform, holding onto the handrail with the hand NOT used to perform swabbing. Once at a convenient and safe height for sampling the rump, transfer template back to ''climbing'' hand (hand used to hold onto the rail while climbing the ladder), taking care not to contaminate the inner edges of the sampling area of the template. Avoid contaminating your sampling hand.

18. Repeat steps 13–15 for the rump area, using the ''clean'' surface or side (the side that was NOT previously used to swab the flank/brisket areas).

19. After swabbing the rump area, carefully place the sponge back in the sample bag, taking care not to touch the outside of the sponge to the outside of the sample bag.

20. While holding the handrail, climb down from the ladder.

21. Expel excess air and fold the top edge of the bag containing the sponge 3 or 4 times to close. Secure the bag by folding the attached wire tie back against the bag.

22. (a) If samples are to be analyzed at an ON-SITE LABORATORY, begin sample preparation (ANALYTICAL METHODS section)

(b) If samples are to be analyzed at an OUTSIDE (OFF-SITE) LABORATORY, follow procedure in the Sample Shipment section.

Swine Surface Sample Collection Procedure

Materials

1. Sterile specimen sponge in sterile Whirl-Pak® bag or equivalent
2. 10 ml sterile Buffered Peptone Water (BPW)
3. Sterile Ziplock-type or stomacher bag
4. Template for a 100 cm^2 sampling area
5. Sterile gloves
6. Wheeled ladder, sampling platform, or step ladder
7. Sanitizing solution
8. Small tote or caddy for carrying supplies

Collection

Read the sections under Pre-sampling Preparation and Preparation for Sample Collection before beginning the sampling procedure. A sterile, moistened sampling sponge (which usually come pre-packaged in a sterile bag) will be used to sample all three sites on the swine carcass (ham, belly, and jowls—see Figure 3). It is important to swab the sampling areas in the order of least to most contaminated to avoid spreading any contamination on the carcass. Therefore, swab sampling areas in the sequence indicated in this protocol. Use predetermined random selection procedures for selecting carcass to be sampled. Remember: samples will be collected from carcasses in the cooler 12 hours or more after slaughter.

Nondestructive surface sampling will be conducted as follows:

1. Ensure that all supplies are on hand. (An assistant may be helpful during the sampling process.)

2. Position the wheeled ladder, sampling platform, or step ladder near the carcass so the ham sample area (Figure 3) is within easy reach from the ladder.

3. Immerse the sampling template in a sanitizing solution for at least 1–2 minutes. Just prior to swabbing the first sampling site on the carcass (step 1), retrieve the sampling template from the hypochlorite sanitizing solution. Shake excess solution from utensil, then protect the portion of the template (especially the inner edges of the sampling area) that will contact the carcass from contamination.

4. Locate the ''belly'', ham, and jowl sampling sites using illustrations and directions in Figure 3 (swine carcass sampling locations).

5. Open the sponge bag by holding the bag at one corner by the wire closure (which is usually colored yellow) then tear off the clear, perforated strip at the top of the bag. (Do not remove or tear off the wire closures). Next, pull apart the two small white tabs on either side of the bag to open the mouth of the bag.

6. Remove cap from sterile BPW tube, being careful not to touch the bottle opening. Carefully pour the entire contents of the BPW bottle (10 ml) into the sponge bag to moisten the sponge.

7. Close the top of the bag by pressing the wire closures together. Use hand pressure from the outside of the bag and carefully massage the sponge until it is FULLY HYDRATED (moistened).

8. With the bag still closed, carefully push the moistened sponge to the upper portion of the bag positioning one narrow end of the sponge up toward the opening of the bag. The whole sponge should still be inside the bag.

9. Open the top of the bag containing the sponge, being careful not to touch the inner surface of the bag with your fingers. The wire closure at the top of

the bag should keep the bag open. Set bag aside.

10. Put on a pair of sterile gloves.

11. Carefully remove the moistened sponge from the bag with your sampling hand. Take care not to touch the surfaces of the sampling sponge intended for sampling with sterile glove.

12. With the other hand, retrieve the template by the outer edge, taking care not to contaminate the inner edges of the sampling area of the template.

13. Locate the ''belly'' sampling area (Figure 2) and place the template over this location.

14. Hold the template in place with one gloved hand (Remember, only the sponge should touch the sampling area. Take care not to contaminate this area with your hands).

15. With the other hand, wipe the sponge over the entire enclosed area (10 cm × 10 cm) for the sample for a total of approximately 10 times in the vertical and 10 times in the horizontal directions. The pressure for swabbing would be as if you were removing dried blood from the carcass. However, the pressure should not be too hard as to crumble or destroy the sponge. (Note: The template may need to be ''rolled'' from side to side during swabbing since the surface of the carcass is not flat. This ensures that the 100 cm^2 area is enclosed while swabbing.)

16. After swabbing the ''belly'' area, transfer the template to the same hand that is holding the sponge. Do not contaminate the inner edges of the sampling area of the template.

17. Climb the ladder or platform, holding onto the handrail with the hand not used for sampling. Once at a convenient and safe height for sampling the ham, transfer template back to the ''climbing'' hand (hand used to hold onto the rail while climbing the ladder), taking care not to contaminate the inner edges of the template. Avoid contaminating your sampling hand.

18. Repeat steps 13–15 for the ham sampling area, using the SAME surface of the sponge used to swab the ''belly'' area.

19. After swabbing the ham area, carefully place the template back to the same hand that is holding the sponge. Do not contaminate the inner edges of the sampling area of the template.

20. While holding the handrail with the hand not used for sampling, climb down from the ladder.

21. Transfer the template back to the ''climbing'' hand (hand used to hold onto the rail while descending the ladder), taking care not to contaminate the inner edges of the template.

22. Repeat steps 13–15 for the the jowl area, using the ''clean'' surface or side (the side that was NOT previously used to swab the ''belly''/ham areas).

23. After swabbing the jowl area, carefully place the sponge back into the sponge bag. Do not touch the surface of the sponge to the outside of the sponge bag.

24. Press wire closures on the sponge bag together, expel the excess air, then fold over the top of the bag 3 or 4 times. Close the bag with attached wire by bending the wire tie back against the bag to secure it.

25. (a) If samples are to be analyzed at an ON-SITE LABORATORY, begin sample preparation (ANALYTICAL METHODS section).

(b) If samples are to be analyzed at an OUTSIDE (OFF-SITE) LABORATORY, follow procedure in the Sample Shipment section.

Whole Chicken Carcass Rinse Sampling Procedure

Materials

1. 2 Sterile 3500 ml stomacher-type bags or equivalent
2. 400 ml sterile Buffered Peptone Water (BPW)
3. Plastic cable-tie wraps or thick rubber bands or equivalent
4. Sterile gloves

Collection

Read the sections under Pre-sampling Preparation and Preparation for Sample Collection before beginning the sampling procedure. Ensure all sampling supplies are present and have been properly labeled. Use predetermined random selection procedure to select a carcass. Birds will be collected after the chiller, at the end of the drip line as follows:

1. Gather all supplies for sampling. An assistant may be helpful during the sampling process when pouring the rinse solution (BPW) into the bag containing the carcass.

2. Put on sterile gloves. Open a stomacher-type 3500 bag without touching the sterile interior of the bag. Rubbing the top edges between the thumb and forefinger will cause the opening to gap for easy opening.

3. With one hand, push up through the bottom of the sampling bag to form a 'glove' over one hand with which to grab the bird, while using your other hand to pull the bag back over the hand that will grab the bird. This should be done aseptically without touching the exposed interior of the bag.

4. Using the hand with the bag reversed over it, pick up the bird by the legs (hocks) through the stomacher bag. (The bag functions as a ''glove'' for grabbing the bird's legs.) Take care not to contaminate the exposed interior of the bag. Allow any excess fluid to drain before reversing the bag back over the bird. (Alternately, have an assistant hold open the bag. Using your gloved hand, pick up the bird by the legs, allow any fluid to drain, and place the bird vent side up into the sampling bag.)

5. Rest the bottom of the bag on a flat surface. While still holding the top of the bag slightly open, add the 400 ml of sterile BPW to the sterile plastic bag. (Alternately, with the aid of an assistant holding the bag open, add the 400 ml of sterile BPW to the bag, pouring the solution into the carcass cavity.)

6. Close the bag and while securely holding the bag, rinse bird inside and out using a rocking motion for 30 shakes (approximately one minute). This is done by holding the bird through the bottom of the bag with one hand and the closed top of the bag with the other hand. Hold the bird securely and rock it in an arcing motion, alternating the weight of the bird from one hand to the other (motion like drawing an invisible rainbow or arch), assuring that all surfaces (interior and exterior of the carcass) are rinsed.

7. Put the bird in the bag on a flat surface. Open the bag.

8. With a gloved hand, remove the carcass from the bag. Since the carcass was rinsed with a sterile solution, it should be returned to the chill tank. Be sure not to touch the interior of the bag with your gloved hand.

9. Twist the top of the bag several times (about 4 or 5 turns). Fold the twisted portion of the bag to form a loop. Secure the twisted loop with the supplied plastic tie-wrap. The tie-wrap should be very tight so that the rinse fluid will not spill out. Place the sample bag into another bag and secure the opening of the outer bag. [Alternately, at least 30 ml of the rinse fluid can be poured into a sterile, leak-proof sampling container and the container then can be placed in a sampling bag for transport to the lab. NOTE: It is important to send at least the minimum volume of rinse fluid, since 30 ml of rinse fluid will be used for sample analysis. The solution remaining after decanting the 30 ml can be poured down the drain]

10. (a) If samples are to be analyzed at an ON-SITE LABORATORY, begin sample preparation for the selected method of analysis.

(b) If samples are to be analyzed at an OUTSIDE (OFF-SITE) LABORATORY, follow the procedure in the Sample Shipment section.

Turkey Carcass Rinse Sampling Procedure

Materials

1. 1 large sterile 3500 ml stomacher-type or ziplock-type bags or equivalent, at least 8″ × 24″
2. 600 ml sterile, Buffered Peptone Water (BPW)
3. Plastic cable-tie wraps or thick rubber bands or equivalent
4. Sterile gloves

Collection

Read the sections under Pre-sampling Preparation and Preparation for Sample Collection before beginning the sampling procedure. Ensure that all supplies are on hand, labeled, and readily available. An assistant will be needed to hold the bag for collecting the bird. Use the predetermined random selection procedure to select the turkey carcass to be sampled. The randomly selected birds will be collected after the chiller, at the end of the drip line as follows:

1. Have an assistant open the large stomacher-type bag (18″ × 24″). (Rubbing the top edges of the stomacher-type bag between the thumb and index finger will cause the opening to gap.) The assistant should be ready to receive the turkey carcass.
2. Put on sterile gloves.
3. Remove the selected turkey from the drip line by grasping it by the legs and allowing any fluid to drain from the cavity.
4. Place the turkey carcass, vent side up, into a sterile Stomacher-type 3500 bag (or equivalent). Large turkeys should be placed in a plain, clear polypropylene autoclave bag (ca. 24″ × 30–36″). Only the carcass should come in contact with the inside of the bag.
5. While still supporting the carcass with one hand on the bottom of the bag, have the assistant open the bag with the other hand. Alternately, the assistant can rest the bottom of the bag on a sanitized table and while still supporting the carcass, open the bag with the other hand.
6. Add the 600 ml of sterile BPW to the sterile plastic bag, pouring the solution into the carcass cavity of the BPW over the exterior of the carcass. Close the bag.
7. Manipulate the loose neck skin on the carcass through the bag and position it over the neck bone area to act as a cushion and prevent puncturing of the bag. The assistant will need to support the carcass with one hand on the bottom of the bag. Close bag.
8. Squeeze air from the bag and close top. Take the bag from the assistant. Close the bag and while securely holding the bag, rinse bird inside and out using a rocking motion for 30 shakes (approximately one minute). This is done by holding the carcass through the bag with one hand and the closed top of the bag with the other hand. Holding the bird securely with both hands, rock in an arcing motion alternating the weight of the bird from one hand to the other (motion like drawing an invisible rainbow or arch), assuring that all surfaces (interior and exterior of the carcass) are rinsed.
9. Hand the bag back to the assistant.
10. With a gloved hand, remove the carcass from the bag first letting any excess fluid drain back into the bag. Since the carcass was rinsed with a sterile solution, it should returned to the chill tank. Be sure not to touch the interior of the bag with your gloved hand.
11. Expel excess air, taking care not to expel any rinse fluid. Twist the top of the bag several times (about 4 or 5 turns). Fold the twisted portion of the bag to form a loop. Secure the twisted loop with the supplied plastic tie-wrap. The tie-wrap should be very tight so that the rinse fluid will not spill out.
12. Place the sample bag into another bag and secure the opening of the outer bag. [Alternately, no less than 30 ml of the rinse fluid can be poured into a sterile, leak-proof sampling container and placed in a sampling bag for transport to the lab. Thirty ml of rinse fluid will be used for sample analysis. The solution remaining after decanting the 30 ml can be poured down the drain]
13. (a) If samples are to be analyzed at an ON-SITE LABORATORY, begin sample preparation for the selected method of analysis. (See Analytical Methods section.)

(b) If samples are to be analyzed at an OUTSIDE (OFF-SITE) LABORATORY, follow the procedure in the Sample Shipment section.

Sample Shipment

It is recommended that samples be analyzed on-site (not in the plant itself, but in a suitable laboratory). Those samples analyzed on-site must be analyzed as soon after collection as possible. If no on-site facilities are available, the samples must be shipped the same calendar day as collected, to an outside laboratory. The samples must be analyzed the day after collection.

1. Prechill shipping container by placing the open shipping container in the refrigerator at least the day before sampling.
2. Place the appropriately-labeled double-bagged sample in the prechilled shipper in an upright position to prevent spillage. Newspaper may be used for cushioning the sample and holding it in the upright position. Ensure that the sample is maintained at refrigeration temperature to prevent multiplication of any microorganisms present and to provide the most accurate results.
3. Place a corrugated cardboard pad on top of the sample. Next, place the frozen gel pack(s) on top of the corrugated pad to prevent direct contact of frozen gel packs with the sample. Use sufficient frozen coolant to keep the sample refrigerated during shipment to the designated laboratory. Insert a foam plug and press it down to minimize shipper head space.
4. Ship sample (via overnight delivery or courier) to the assigned laboratory.

Analytical Methods

Equipment, Reagents, and Media

Equipment

1. Sterile scalpels, scissors, forceps, knives, spatulas, spoons, ruler or template, pipettes, petri dishes, test tubes
2. Sterile Stomacher 3500 bags (or equivalent) or plain, clear polypropylene autoclave bags (ca. 24″ × 30–36″)
3. Incubator, 36 ± 1°C
4. Incubator/Water bath, 42 ± 0.5°C
5. A mechanical homogenization device. A Stomacher, used with sterile plastic bags, is acceptable. Some laboratories prefer to use a sterile Osterizer-type blender with sterilized cutting assemblies and adapters for use with sterile Mason jars.
6. Water bath, 48–50°C
7. Glass slides, glass plate marked off in one-inch squares or agglutination ring slides
8. Balance, 2000 gram capacity, sensitivity of 0.1 gram
9. Inoculating needles and loops
10. Vortex mixer
11. Sterile sampling sponge and sponge bag

Reagents

1. Iodine solution for TT broth (Hajna)
2. Buffered Peptone Water (BPW) diluent
3. Methyl red reagent
4. O'Meara's V–P reagent, modified
5. Kovac's reagent
6. Ferric chloride, 10% aqueous solution
7. Sterile mineral oil
8. Saline, 0.85%
9. Saline, 0.85% with 0.6% formalin
10. *Salmonella* polyvalent O antiserum
11. *Salmonella* polyvalent H antiserum
12. *Salmonella* individual O grouping sera for groups A–I

Media

1. Buffered peptone water (BPW)
2. Tetrathionate broth (TT-Hajna)
3. Rappaport-Vassiliadis (RV) broth (4)—Merck Chemical Co., Cat. #7700 or equivalent
4. Brilliant green sulfa agar (BGS; contains 0.1% sodium sulfapyridine)
5. Double modified lysine iron agar (DMLIA; 2)
6. Triple sugar iron agar (TSI)
7. Lysine iron agar (LIA)
8. MR–VP Medium
9. Tryptone broth
10. Simmons citrate agar
11. Phenol red tartrate agar
12. Motility Medium
13. Christensen's urea agar
14. Carbohydrate fermentation media with Andrade's indicator
15. Decarboxylase test media (Moeller)
16. Malonate broth
17. KCN broth
18. Phenylalanine agar
19. Nutrient gelatin
20. Trypticase soy broth
21. Tryptose broth

Analytical Procedures

Sample Preparation for Analysis

The diverse nature of the samples which may require analysis (e.g., ground product versus a poultry carcass rinse sample) requires separate preparation procedures for each sample type.

Raw Ground Product Sample Preparation

a. Use a sterile spoon or spatula to take portions of product from several areas of the sample to prepare a 25 g composite sample in a sterile plastic stomacher-type bag or blender jar. Use of a stomacher filter bag may facilitate pipetting after pre-enrichment.

b. Add 225 ml BPW. Homogenize for two minutes in a Stomacher or blender.

Beef or Pork Carcass Sponge Sample Preparation

a. Add 50 ml of BPW to the sample bag containing the sponge to bring the total volume to 50 ml. Mix well.

Whole Chicken Carcass Rinse-Fluid Sample Preparation

a. Remove 30 ml of carcass-rinse fluid and place it in a sterile plastic bag or other sterile container.

b. Add 30 ml of BPW to the sample. Mix well.

Turkey Carcass Rinse-Fluid Sample Preparation

a. Remove 30 ml of carcass-rinse fluid and place it in a sterile plastic bag or other sterile container.

b. Add 30 ml of BPW to the sample. Mix well.

Detection Procedure

Sample/BPW suspensions prepared as directed in Sample preparation for analysis section (above) are the starting point for this step in the protocol. From this point on, sample suspensions of various types (e.g., whole bird rinse sample vs. raw ground product) can be treated in the same manner.

Note: If using a screening test, follow manufacturer's instruction for enrichment procedures. If an alternate enrichment scheme is to be used, verification of the effectiveness of this alternate enrichment protocol with the screening test should be received from the manufacturer of the screening test or by in-laboratory testing.

1. Incubate sample/BPW suspension at 36 ± 1°C for 20–24 hours.

2. a. Transfer 0.5 ml of the BPW sample pre-enrichment culture into 10 ml TT broth.

b. Transfer 0.1 ml of the BPW sample pre-enrichment culture into 10 ml RV broth.

3. a. Incubate the TT enrichment culture at 42 ± 0.5°C for 22–24 hours.

b. Incubate the RV enrichment culture at 42 ± 0.5°C for 22–24 hours.

4. Streak each enrichment culture onto both DMLIA and BGS agar plates. Do not subdivide plates for streaking multiple samples; streak the entire agar plate with a single sample enrichment.

5. Incubate plates at 36 ± 1°C.

6. Examine plates after 22–24 hours of incubation. Reincubate negative plates and reexamine them the following day.

7. Select and confirm suspect colonies as described in the sections for Isolation procedure through Biochemical testing procedures (below).

Isolation Procedure

1. Pick typical well-isolated colonies.

a. BGS. Select colonies that are pink and opaque with a smooth appearance and an entire edge surrounded by a red color in the medium. On very crowded plates, look for colonies that appear tan against a green background.

b. DMLIA. Select purple colonies with or without black centers. Since salmonellae typically decarboxylate lysine and ferment neither lactose nor sucrose, the color of the medium reverts to purple.

2. Select three suspect colonies from each plate. Pick only from the surface and center of the colony. Avoid touching the agar because these selective media may suppress growth of organisms which are viable but not visible; such ''sleeper'' organisms can be picked up from the agar surface and carried forward onto media used for confirmation tests. If a plate is crowded and there are no well-isolated colonies available, restreak from this plate *directly* onto fresh selective agar plates.

Initial Isolate Screening Procedure

1. Inoculate TSI and LIA slants consecutively with a single pick from a colony by stabbing the butts and streaking the slants in one operation. If screw-cap tubes are used, the caps must be loosened before incubation. Incubate at 36 ± 1°C for 24±2 hours.

2. Examine TSI and LIA slants as sets. Note the colors of butts and slants, blackening of the media and presence of gas as indicated by gas pockets or cracking of the agar. Note also the appearance of the growth on the slants along the line of streak. Discard sets that show ''swarming'' from the original site of inoculation. Discard sets that show a reddish slant in LIA. Isolates giving typical *Salmonella* spp. reactions should be confirmed by serological tests. Examine isolates which are suggestive, but not typical of *Salmonella* spp. by a combination of biochemical and serological procedures. Confirm by biochemical tests ONLY those isolates that appear typical of *salmonellae,* but do not react serologically. Refer to the following chart for assistance in making these determinations.

Triple sugar iron agar			Lysine iron agar		Polyvalent sera		Disposition
Butt	Slant	H_2S	Butt	H_2S	O	H	
Y	R	+	P	+	+	+	Salmonella spp.
Y	R	+	P	+	+	-	B. & M. T.
Y	R	-	P	-	.....	.....	B. & M. T.
Y	R	-	Y	-	+	+	B. & M. T.[1]
Y	R	-	Y	-	-	.....	Discard.
Y	R	+	Y	±	.....	.....	Discard.
Y	Y	-	Y/P	-	.....	.....	Discard.

Triple sugar iron agar			Lysine iron agar		Polyvalent sera		Disposition
Butt	Slant	H_2S	Butt	H_2S	O	H	
Y	Y	+	P	+	.....	.....	B. & M. T.[2]
NC	NC	.....	.....	.....	.....	.....	Discard.

Y = Yellow; R = Red; P = Purple; B. & M. T. = Biochemical and motility tests; NC = No change in color from uninoculated medium.
[1] *Salmonella choleraesuis* (rarely found in swine in U.S.).
[2] *Salmonella arizonae.*

Serological Tests

All isolates giving TSI and LIA reactions which could be considered suggestive of *Salmonella* should be tested serologically. If the TSI and LIA reactions, together with the serological reactions, are indicative of *Salmonella,* confirmation may cease at this point. If, however, atypical TSI or LIA results and/or negative serological tests are encountered, biochemical testing is mandatory (see Biochemical testing procedure, below).

1. O Agglutination Tests

At a minimum, isolates should be tested with polyvalent O antiserum reactive with serogroups A through I. Following a positive reaction with polyvalent O antiserum, it is necessary to type the isolate using individual *Salmonella* antisera for O groups A through I. Testing for O groups A through I should encompass the majority of the *Salmonella* serotypes commonly recovered from meat and poultry products. Occasionally, however, an isolate which is typical of *Salmonella* (biochemically and Poly H serologically) but non-reactive with antisera to groups A through I will be recovered; such an isolate should be reported as ''*Salmonella* non A–I'' or ''*Salmonella* O group beyond I''.

Follow the manufacturer's instructions enclosed with the antisera. Use growth from either the TSI or LIA slant. Test the isolate first using polyvalent O antiserum. Do not read agglutination tests with a hand lens. If there is agglutination with the saline control alone (autoagglutination), identify such an isolate by biochemical reactions. If the saline control does not agglutinate and the polyvalent serum does, identify the individual O group using the individual Salmonella O grouping antisera for groups A through I. Record positive results and proceed to H agglutination tests.

2. H Agglutination Tests

Inoculate Trypticase soy broth or Tryptose broth. Incubate at 36 ± 1 °C overnight or until growth has an approximate density of three on McFarland's scale. Add an equal amount of saline containing 0.6% formalin and let set one hour. Remove one ml to each of two 13 × 100 mm test tubes. To one of the tubes, add *Salmonella* polyvalent H serum in an amount indicated by the serum titer or according to the manufacturer's instructions. The other tube serves as an autoagglutination control. Incubate both tubes at 48–50 °C in a water bath for up to one hour. Record presence or absence of agglutination. Alternatively, any other poly H agglutination test may be used as long as it gives results equivalent to the conventional tube agglutination procedure described above.

Biochemical Testing Procedures

Biochemical confirmation is only necessary with those isolates giving atypical TSI or LIA results and/or negative serological tests. Do the minimum number of tests needed to establish that an isolate can be discarded or that it is a member of the genus *Salmonella*. Exhaustive testing of any isolate from a sample that has already yielded a typical, easily identifiable *Salmonella* is unnecessary.

If further testing is necessary, inoculate the following media first: Tryptone broth, MR–VP medium, Simmons citrate agar, Christensen's urea agar, motility test medium, phenol red tartrate agar, and glucose, lactose, sucrose, salicin and dulcitol fermentation broths. Incubate at 36 ± 1 °C and record reactions the following day. Test Tryptone broth with Kovac's reagent for indole production in 24 hours and, if negative, again in 48 hours. Do not perform the MR–VP test until 48 hours have elapsed. If results are ambiguous, repeat MR test after five days of incubation. Hold negative carbohydrate fermentation tests for 14 days.

Refer to ''Edwards and Ewing's Identification of Enterobacteriaceae'', 4th Edition (3), for biochemical reactions of Enterobacteriaceae and for fermentation media and test procedures.

Discard all isolates that give positive urea or VP reactions. Discard any isolate that has the following combination of characteristics: produces gas in glucose, produces indole but not H_2S, is MR positive, VP negative and citrate negative; such organisms are E. coli regardless of ability to ferment lactose in 48 hours.

Inoculate additional biochemical tests as necessary to eliminate other Enterobacteriaceae. Refer to Edwards and Ewing for details. Eliminate Providencia spp. by a positive phenylalanine reaction. Eliminate Hafnia alvei on the basis of the following biochemical pattern: indole negative; MR negative, and VP and citrate positive based on four days of incubation at 25 °C; fermentation of arabinose and rhamnose; failure to ferment adonitol, inositol, sorbitol, and raffinose.

Alternatively, any other biochemical test system may be used as long as it gives results equivalent to the conventional tests.

Quality Control Procedures

It is recommended that a minimum of three method controls be analyzed whenever meat or poultry products are being examined for the presence of salmonellae. These controls should include a S. typhimurium (H_2S positive), S. senftenberg (H_2S negative), and an uninoculated media control. The inoculum level for the positive controls should approximate 30–300 CFU per container of enrichment medium. Inoculate positive controls at the end of each day's run. Incubate the three controls along with the samples, and analyze them in the same manner as the samples. Confirm at least one isolate recovered from each positive control sample.

Storage of Isolates

Do not store isolates on TSI agar because this tends to cause roughness of O antigens. For short-term (2–3 months) storage, inoculate a nutrient agar slant, incubate at 36 ± 1 °C overnight, and then store at 4–8 °C.

For long-term storage of isolates, subculture Salmonella isolates by stabbing nutrient agar (0.75% agar). Incubate at 36 ± 1 °C overnight, and then seal with hot paraffin-soaked corks. Household wax is better than embedding paraffin because it stays relatively soft at room temperature making the corks easy to remove. Store isolates *in the dark at room*

temperature. Such isolates will remain viable for several years.

Store ''working'' Salmonella stock cultures on nutrient agar slants. Transfer stocks monthly, incubate overnight at 36 ± 1 °C, and then store them at 4–8 °C.

References

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2. Bailey, J. S., J. Y. Chiu, N.A. Cox, and R.W. Johnston. 1988. Improved selective procedure for detection of salmonellae from poultry and sausage products. J. Food Protect. 51(5):391–396.

3. Ewing, W. H. 1986. ''Edwards and Ewing's Identification of Enterobacteriaceae'', 4th Edition. Elsevier Science Publishing Co., Inc., New York, NY.

4. Vassiliadis, P. 1983. The Rappaport-Vassiliadis (RV) enrichment medium for the isolation of salmonellas: An overview. J. Appl. Bacteriol. 54:69–76.

BILLING CODE 3410–DM–P

Figure 1. Example of sampling template (not drawn to scale)

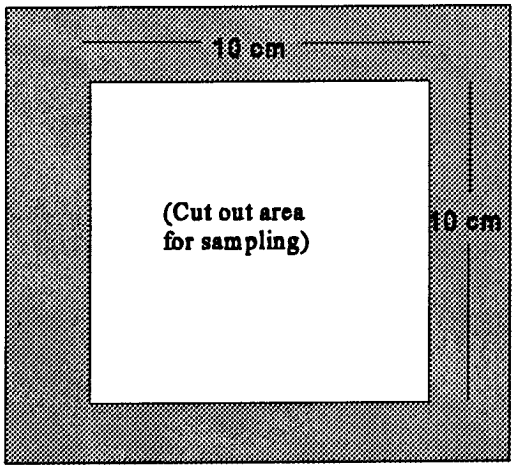
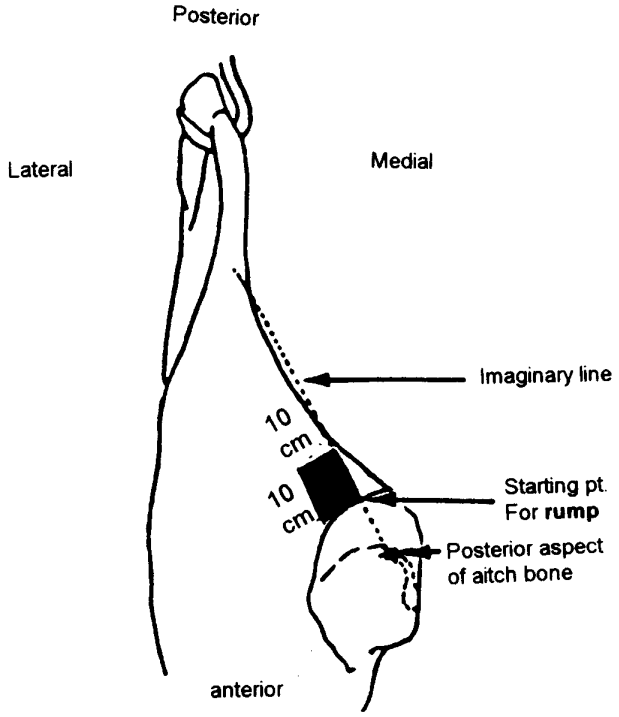


Figure 2. Sampling locations for *Salmonella* testing of cattle carcasses

Rump Locate the posterior aspect of the aitch bone. Draw an imaginary line toward the achilles tendon. At the point where the line intersects the cut surface of the round is the starting point for the rump sample. Measure 10 cm up the line leading to the achilles tendon, then 10 cm over (laterally), then 10 cm back to the cut surface of the round, then 10 cm along the cut surface to form the 10 cm by 10 cm square area.

Note: This upper illustration has been purposely altered somewhat. A true lateral view of the carcass would not show the aitch bone. From a medial view, the whole 10 cm x 10 cm sampling area could not be seen. Therefore, a lateral view with a portion of the round removed so the location of the aitch bone is shown is illustrated.



Flank Locate the cutaneous flank muscle (external abdominal oblique) and follow the medial border of the muscle anteriorly until it comes with approximately 3" of the midline. This will be the starting point. Measure up (posteriorly) 10 cm (approximately 4 inches) along a line approximately 3" from the midline (measure up or parallel to the midline), then over (laterally) 10 cm (approximately 4 inches) to form a 10 cm wide by 10 cm long square sample.

Brisket Locate the elbow of the carcass. Draw an imaginary line straight across (medially) to the midline cut. This will be the starting point. Measure up along the midline 10 cm (approximately 4 inches), then over 10 cm (approximately 4 inches) to form a 10 cm wide by 10 cm long square sample.

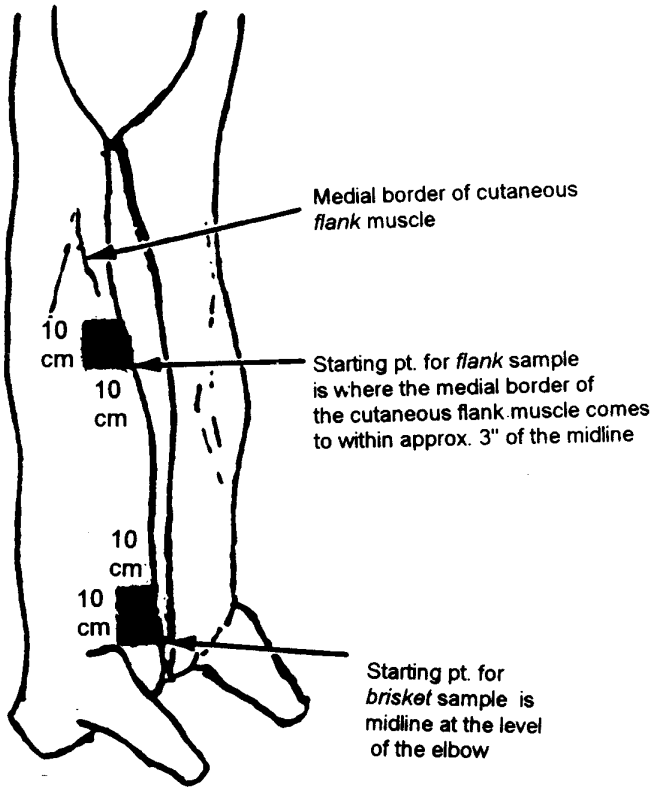
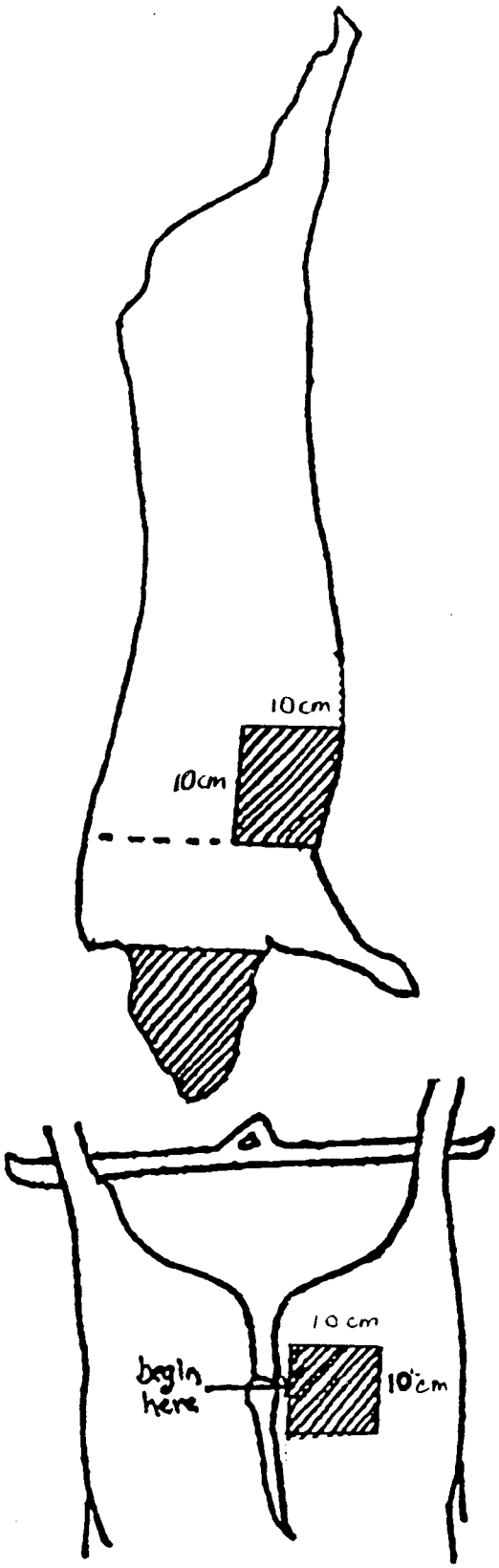


Figure 3. Sampling locations for *Salmonella* testing of swine carcasses



belly Locate the elbow of the carcass. Draw an imaginary line straight across (medially) to the midline cut. This will be the starting point. Measure up along the midline 10 cm (approximately 4 inches), then over 10 cm (approximately 4 inches) to complete the 10 cm long by 10 cm wide square sample area to swab for swine "belly" sample.

jowls Draw an imaginary line from the atlas/axis joint to the ventral midline; all skin below that point will be considered the jowl.

ham From the dorsal position, locate the lateral surface of the base of the tail, and measure up (caudal) 5 cm along the lateral edge of the exposed fat margin, then 10 cm laterally. Now measure 10 cm down (cranial), then 10 cm medially, then 5 cm up (posteriorly) to complete a 10 cm long by 10 cm wide square sampling area.

BILLING CODE 3410–DM–C

Appendix F—Guidelines for Escherichia coli Testing for Process Control Verification in Cattle and Swine Slaughter Establishments

Introduction

Under the Pathogen Reduction/HACCP Regulation, all slaughter establishments will be required to test carcasses for generic *E. coli* as a tool to verify process control. This document outlines the sampling and microbial testing that should be followed to meet this requirement. It also gives guidance to interpreting your results. This document is a supplement to the Regulation, but not a substitute for it. Further in-depth details of the program may be found in the Regulation. Please provide these guidelines to your company microbiologist or testing laboratory in order to help you meet the regulatory requirements for generic *E. coli* testing.

Guidelines for Sample Collectors/Microbiologists

Background

This sampling protocol has been prepared to support the Pathogen Reduction/HACCP Regulation. This protocol incorporates the use of a nondestructive sampling technique for sample collection from raw beef and swine carcasses. These techniques have been evaluated by the Agricultural Research Service and have been designed to give comparable results to the FSIS Nationwide Microbiological Baseline Data Collection Programs' excised tissue samples. We are continuing to improve the sponging techniques and welcome comments. This technique will also be used in the FSIS *Salmonella* testing programs and will be closely monitored during the first year of prevalence phase testing.

Carcasses within the same establishment and in different establishments must be sampled and analyzed in the same manner if the results are to provide a useful measure of process control across the nation. It is imperative that all like establishments adhere to the same sampling and analysis requirements detailed here, without deviation. These sampling and analytical procedures may be directly written into your establishment's individual HACCP plan.

Cattle and swine carcasses must be sampled at the end of the slaughter process in the cooler. These sample collection locations are the same as those in the FSIS baseline studies, making samples taken here comparable to the nationwide baseline performance criteria.

Pre-sampling Preparation

Sample collection will be carried out by the individual designated in the establishment's written protocol for microbiological sampling. This protocol should include a check list of tasks to be performed prior to sample collection, materials needed for sample collection, random selection procedures, where the samples will be analyzed (on-site versus off-site), and other information that will aid the sample collector. As stated previously, this guideline can be a part of the plant's sample collection guidelines, but plant specific details and procedures will need to be included. Sampling supplies, such as sterile gloves, sterile sampling solutions, hand soap, sanitizing solution, etc., as well as specific materials needed for sampling different carcass types (i.e., specimen sponges in bags and template for sampling cattle or swine carcasses), will need to be assembled prior to beginning sample collection.

For cattle and swine carcass sampling, a template will be needed to mark off the area to sample. The template can be made of metal or aluminum foil, brown paper, flexible plastic, etc. Some disposable templates may come sterilized and individually prepackaged. To make a reusable template, cut out a 10 centimeters (cm) x 10 cm (3.94 inches x 3.94 inches) square from a sheet larger than the area to be sampled. (See Figure 1). If a reusable template is used, it will need to be sanitized with an approved sanitizing solution [e.g., hypochlorite (bleach) solution or alcohol]. However, the template needs to be dry before placing it on the carcass. Aluminum foil or paper templates can be used once and discarded. The foil for the template should be stored in a manner to prevent contamination. Since the area enclosed by the template will be sampled, take care not to touch this area with anything other than the sampling sponge. Using dirty or contaminated material may lead to erroneous results. If an autoclave is available, paper or aluminum foil templates can be wrapped in autoclavable paper and sterilized.

Sterile sampling solutions, Butterfield's phosphate diluent (BPD), can be stored at room temperature. However, at least on the day prior to sample collection, check solutions for cloudiness. DO NOT use solutions that are cloudy, turbid or contain particulate matter. Place the number of containers of sampling solution (BPD) that will be needed for the next day's sampling in the refrigerator.

To obtain the most accurate results, samples should be analyzed as soon after collection as possible. However, if samples must be transported to an off-site laboratory, the samples need to be maintained at refrigeration temperatures until transport, then shipped refrigerated via an overnight delivery service to the laboratory performing the analysis. Samples analyzed off-site must be picked up by the overnight courier the SAME calendar day the sample is collected. The sample must arrive at the laboratory the day after the sample is collected. Samples shipped to an outside laboratory must be analyzed no later than the day after collection. The following section gives information on shipping containers and transporting samples to off-site facilities.

Shipping Containers and Coolant Packs

It is important that samples fit easily into the shipping containers so that the sample bags do not break. Correct use of the refrigerant gel-ice packs and proper packing of the shipping container are necessary so that samples arrive at the laboratory at an acceptable temperature. Frozen samples or samples which are too warm are not considered valid and must not be analyzed. Some bacteria may be damaged by temperatures that are too cold, while temperatures that are too warm can allow bacteria to reproduce. Maintaining samples at improper temperatures may cause inaccurate sample results. The sample should be kept refrigerated, NOT FROZEN, in the shipping container prior to pickup by the courier service. The shipping container, itself, should not be used as a refrigerator. However, multiple samples (if needed) for that day may be stored in the open shipping container in the cooler or refrigerator.

Sampling frequency

Sampling frequency for *E. coli* testing is determined by production volume. The required minimum testing frequencies for all but very low production volume establishments are shown in Table 1 by slaughter species.

TABLE 1.—E. COLI TESTING FREQUENCIES [a]

Cattle	1 test per 300 carcasses.
Swine	1 test per 1,000 carcasses.

[a] Note: These testing frequencies do not apply to very low volume establishments. See Table 2.

Very Low Volume Establishments

Some establishments may be classified as very low volume establishments. The maximum yearly

slaughter volumes for very low volume establishments are described in Table 2.

TABLE 2.—MAXIMUM YEARLY LIVESTOCK SLAUGHTER VOLUMES FOR VERY LOW VOLUME ESTABLISHMENTS

Slaughter species	Criteria (yearly slaughter volume)
Cattle	Not more than 6,000 head.
Swine	Not more than 20,000 head.
Cattle and Swine.	Not more than 20,000 total, with not more than 6,000 cattle.

Establishments with very low volumes are to sample the predominant species at an initial rate of once per week until at least 13 test results have been obtained. Once the initial criteria have been met for very low volume establishments (see APPLYING PERFORMANCE CRITERIA TO TEST RESULTS), the establishment will repeat the same sampling regime once per year, in the 3 month period of June through August, or whenever a change is made in the slaughter process or personnel.

Random Selection of Carcasses

Samples are to be taken randomly at the required frequency (See section on Sampling Frequency). For example, given the frequency of testing for cattle is 1 (one) test per every 300 cattle slaughtered, then if a plant slaughters 150 head of cattle an hour, 1 (one) sample will be taken every 2 hours.

Different methods of selecting the specific carcass for sampling could be used, but all require the use of random numbers. Methods could include: using random number tables, using calculator- or computer-generated random numbers, drawing cards, etc. When selecting the random numbers, use the method(s) currently in use at the establishment for other sampling programs, if other programs are currently underway.

The carcass for sampling must be selected at random from all eligible carcasses. If multiple lines exist, randomly select the line for sample collection for that interval. Repeat the random selection process for the next sampling interval. Each line should have an equal chance of being selected at each sampling interval.

Cattle Carcass Selection

The half-carcasses eligible for sampling should be selected from those in the cooler 12 or more hours after slaughter. Both the ''leading'' and ''trailing'' sides of a carcass should have an equal chance of being selected within the designated time frame (based on the sampling frequency for the plant). NOTE: If more than one shift is operating at the plant, the sample can be taken on any shift, provided the following requirements are met:

Selection of TIME: Select the time, based on the appropriate sampling frequency, for collecting the sample.

Selection of COOLER SITE: Select a safe and accessible site in the cooler for random selection of the half-carcass. This site may be located at the transfer chain, grading chain, or a rail that contains carcasses that have been chilled 12 hours or more. If there are multiple sites of the same kind, select one at random.

Selection of HALF-CARCASS: Based on the sampling frequency for the plant, identify a half-carcass (selected by your random number method) from the predetermined point along the chain (cooler site) and then count back five (5) half-carcasses and select the next half-carcass (carcass) for sampling. The reason for counting back five half-carcasses is to avoid any possible bias during selection. (See Sampling Frequency section to determine the rate of sampling.)

Swine Carcass Selection

The carcasses eligible for sampling should be selected from those in the cooler 12 or more hours after slaughter. Every carcass should have an equal chance of being selected within the designated time frame (based on the sampling frequency for the plant). NOTE: If more than one shift is operating at the plant, the sample can be taken on any shift, provided the following requirements are met:

Selection of TIME: Select the time, based on the appropriate sampling frequency, for collecting the sample.

Selection of COOLER SITE: Select a safe and accessible site in the cooler for random selection of the carcass. This site may be located at the transfer chain, grading chain, or a rail that contains carcasses that have been chilled 12 hours or more. If there are multiple sites of the same kind, select one at random.

Selection of CARCASS: Based on the sampling frequency for the plant, identify a whole carcass from the predetermined point along the chain and then count back five (5) carcasses and select the next carcass for sampling. The reason for counting back five carcasses is to avoid any possible bias during selection. (See Sampling Frequency section to determine the rate of sampling.)

Aseptic Techniques/Sampling

Extraneous organisms from the environment, hands, clothing, sample containers, sampling devices, etc., may lead to erroneous analytical results. More stringent requirements for microbiological analysis are necessary, therefore, use of aseptic sampling techniques and clean, sanitized equipment and supplies are of utmost importance.

There should be an area designated for preparing sampling supplies, etc. A stainless steel, wheeled cart or table would be useful during sampling. A small tote or caddy could be moved to the location of sampling and could be used for carrying supplies, supporting sample bags when adding sterile solutions to sample bags, etc.

Sterile gloves should be used for collecting samples. The only items which may contact the external surface of the glove are the exposed sample being collected and/or the sterile sample utensil (specimen sponge). Keep in mind that the outside surfaces of the sample container are not sterile. Do not handle the inside surface of the sterile sample containers. Do not touch anything else. The following procedure for putting on sterile gloves can be followed when collecting samples:

(a) Peel open the package of sterile gloves from the top without contaminating (touching, breathing on, contacting, etc.) the exterior of the gloves.

(b) Remove a glove by holding it from the wrist-side opening inner surface. Avoid any contact with the outer surface of the glove. Insert the washed and sanitized hand into the glove, taking care not to puncture the glove.

(c) Taking care not to contaminate the exterior surface of the glove, repeat the above step for the hand you will use to physically handle the sample.

(d) If at any time you are concerned that a glove may be

Preparation for Sample Collection

Prior to collecting samples, review appropriate sampling steps, random selection procedures, and other information that will aid in sample collection.

On the day prior to sample collection, after checking for cloudiness/turbidity, place the number of BPD containers that will be needed for the next day's sampling in the refrigerator/cooler. If samples are to be shipped to an off-site facility, pre-chill shipping container and refrigerator packs.

On the day of sampling, gather all sample collection bags, sterile gloves, sanitizer, hand soap, sterile solutions for

sampling, and specific materials listed under the Materials section of the sample collection section for the type of carcass to be sampled. Ensure that all sampling supplies are on hand and readily available before beginning sample collection.

Label the sample bags before starting the sampling procedure. Use permanent ink. If you are using paper labels, it is important that the label be applied to the bag at normal room temperature; it will not stick if applied in the cooler.

Outer clothing (frocks, gloves, head gear, etc.) worn in other areas of the plant should be removed before entering the sampling area or preparing to collect samples. Replace outer clothing removed earlier with clean garments (i.e., laboratory coat) that have not been directly exposed to areas of the plant outside of the sampling area.

Sanitize the sample work area surfaces by wiping with a clean disposable cloth or paper towel dipped in a freshly prepared 500 ppm (parts per million) sodium hypochlorite solution (0.05% sodium hypochlorite) or other approved sanitizer which provides an equivalent available chlorine concentration. The sample work area surfaces must be free of standing liquid before sample supplies and/or product containers are placed on them.

Before sampling, thoroughly wash and scrub hands to the mid-forearm. Use antibacterial hand soap. If available, this should include a sanitizer at 50 ppm equivalence available chlorine. Dry the hands using disposable paper towels.

Specific Sample Collection Procedures

Cattle Sample Collection Procedure

Materials

1. Sterile specimen sponge in sterile Whirl-pack®-type bag or equivalent
2. 25 ml sterile Butterfield's phosphate diluent (BPD)
3. Sterile ziplock-type or stomacher bag
4. Template for 100 cm^2 sampling area
5. Sterile gloves
6. Wheeled ladder, sampling platform, or step ladder
7. Sanitizing solution
8. Small tote or caddy for carrying supplies

Collection

Read the sections under Pre-sampling Preparation and Preparation for Sample Collection before beginning the sampling procedure. Use predetermined random selection procedures for selecting the half-carcass to be sampled. Remember, samples will be collected from half-carcasses in the cooler 12 hours or more after slaughter.

A sampling sponge (which usually comes dehydrated and prepackaged in a sterile bag) will be used to sample all three sites on the carcass (flank, brisket, and rump—see Figure 2). It is important to swab the areas in the order of least to most contamination in order to avoid spreading any contamination.

Therefore, swab the areas in the sequence indicated in this sampling protocol. Nondestructive surface sampling will be conducted as follows:

1. Ensure that all bags have been pre-labeled and all supplies are on hand, including the sampling template. (An assistant may be helpful during the sampling process.)
2. IF a reusable template is used, immerse the sampling template in an approved sanitizing solution for at least 1–2 minutes. Just prior to swabbing the first sample site on the carcass (step 13), retrieve the sampling template from the sanitizing solution. Shake excess solution from the utensil, then protect the portion of the template that will contact the carcass from contamination.
3. Locate the flank, brisket, and rump sampling sites using illustrations and directions in Figure 2 (cattle carcass sampling locations).
4. Position the wheeled ladder, sampling platform, or step ladder near the carcass so the rump sample area (Figure 2) is within easy reach from the ladder.
5. While holding the sponge bag at the top corner by the wire closure, tear off the clear, perforated strip at the top of the bag.
6. Remove the cap from sterile BPD bottle, being careful not to touch the bottle opening.
7. Carefully pour about half the contents of the sterile BPD bottle (approximately 10 ml) into the sponge bag to moisten the sponge.
8. Close the top of the bag by pressing the wire closures together. Use hand pressure from the outside of the bag and carefully massage the sponge until it is FULLY HYDRATED (moistened).
9. With the bag still closed, carefully push the moistened sponge to the upper portion of the bag orienting one narrow end of the sponge up toward the opening of the bag. Do NOT open the bag or touch the sponge with your fingers. While holding the bag, gently squeeze any excess fluid from the sponge using hand pressure from the outside. The whole sponge should still be in the bag.
10. Open the bag containing the sponge, being careful not to touch the inner surface of the bag with your fingers. The wire closure at the top of the bag should keep the bag open. Set bag aside.
11. Put on a pair of sterile gloves.
12. Carefully remove the moistened sponge from the bag with the thumb and fingers (index and middle) of your sampling hand.
13. With the other hand, retrieve the template by the outer edge, taking care not to contaminate the inner edges of the sampling area of the template.
14. Locate the flank sampling area (Figure 2). Place the template over this location.
15. Hold the template in place with one gloved hand (Remember, only the sponge should touch the sampling area. Take care not to contaminate this area with your hands)
16. With the other hand, wipe the sponge over the enclosed sampling area (10 cm x 10 cm) for a total of approximately 10 times in the vertical and 10 times in the horizontal directions. The pressure for swabbing would be as if you were removing dried blood from the carcass. However, the pressure should not be too hard as to crumble or destroy the sponge. (Note: The template may need to be ''rolled'' from side to side during swabbing since the surface of the carcass is not flat. This ensures that the 100 cm^2 area is enclosed while swabbing.)
17. Repeat steps 14–16 for the brisket area, using the SAME side or surface of the sponge used to swab the flank area.
18. After swabbing the brisket area, transfer the template to the same hand holding the sponge. Do not contaminate the sponge or inner edges of the sampling area of the template.
19. Climb the ladder or platform, holding onto the handrail with the hand used to hold the template. Once at a convenient and safe height for sampling the rump, transfer template back to ''climbing'' hand (hand used to hold onto the rail while climbing the ladder), taking care not to contaminate the inner edges of the template.
20. Repeat steps 14–16 for the rump area, using the ''clean'' surface or side (the side that was NOT previously used to swab the flank/brisket areas) of the sponge.
21. After swabbing the rump area, carefully place the sponge back in the sponge sample bag, taking care not to touch the sponge to the outside of the sample bag.
22. While holding the handrail, climb down from the ladder.
23. Add the additional BPD (about 15 ml) to the sample bag to bring the total volume to approximately 25 ml.
24. Expel excess air from the bag containing the sponge and fold down the top edge of the bag 3 or 4 times to close. Secure the bag by folding the attached wire tie back against the bag.

Place closed sponge bag into second bag and close the second bag securely.

25. (a) If samples are to be analyzed at an ON-SITE LABORATORY, begin sample preparation (ANALYTICAL METHODS section)

(b) If samples are to be analyzed at an OUTSIDE (OFF-SITE) LABORATORY, follow procedure in the Sample Shipment section.

Swine surface sample collection procedure:

Materials

1. Sterile specimen sponge in sterile Whirl-Pak®-type bag or equivalent
2. 25 ml sterile Butterfield's phosphate diluent (BPD)
3. Sterile ziplock-type or stomacher-type bag
4. Template for a 100 cm^2 sampling area
5. Sterile gloves
6. Wheeled ladder, sampling platform, or step ladder
7. Sanitizing solution
8. Small tote or caddy for carrying supplies

Collection

Read the sections under Pre-sampling Preparation and Preparation for Sample Collection before beginning the sampling procedure. Use predetermined random selection procedures for selecting carcass to be sampled. Remember: samples will be collected from carcasses in the cooler 12 hours or more after slaughter. A sampling sponge (which usually comes dehydrated and prepackaged in a sterile bag) will be used to sample all three sites on the swine carcass (belly, ham, and jowl—see Figure 3). It is important to swab the areas in the order of least to most contamination in order to avoid spreading any contamination. Therefore, swab the areas in the sequence indicated in this sampling protocol. Nondestructive surface sampling will be conducted as follows:

1. Ensure that all supplies are on hand. (An assistant may be helpful during the sampling process.)

2. If a reusable template is used, immerse the sampling template in a sanitizing solution for at least 1–2 minutes. Just prior to swabbing the first sample site on the swine carcass (step 12), retrieve the sampling template from the sanitizing solution. Shake excess solution from the utensil, then protect the portion of the template that will contact the carcass from contamination.

3. Locate the belly, ham, and jowl sampling sites using illustrations and directions in Figure 3 (swine carcass sampling locations).

4. Position the wheeled ladder, sampling platform, or step ladder near the carcass so the ham sample area (Figure 3) is within easy reach from the ladder.

5. Hold the sponge bag at the top corner by the wire closure, then tear off the clear perforated strip at the top of the bag. Open the bag.

6. Remove the cap from sterile BPD bottle, being careful not to touch the bottle opening. Do not contaminate the lid.

7. Carefully pour about half of the contents of the sterile BPD bottle (10 ml) into the sponge bag to moisten the sponge. Put the lid back on the BPD bottle.

8. Close the top of the bag by pressing the wire closures together. Use hand pressure from the outside of the bag and carefully massage the sponge until it is FULLY HYDRATED (moistened).

9. With the bag still closed, carefully push the moistened sponge to the upper portion of the bag orienting one narrow end of the sponge up toward the opening of the bag. Do NOT open the bag or touch the sponge with your fingers. While holding the bag, gently squeeze any excess fluid from the sponge using hand pressure from outside. The whole sponge should still be inside the bag.

10. Open the bag containing the sponge, being careful not to touch the inner surface of the bag with your fingers. The wire closure at the top of the bag should keep the bag open.

11. Put on a pair of sterile gloves.

12. Carefully remove the moistened sponge from the bag with the thumb and fingers (index and middle) of your sampling hand.

13. With the other hand, retrieve the template by the outer edge, taking care not to contaminate the inner edges of the sampling area of the template.

14. Locate the belly sampling area (Figure 2). Place the template over this location.

15. Hold the template in place with one gloved hand. Remember, only the sponge should touch the sampling area. Take care not to contaminate this area with your hands.

16. With the other hand, wipe the sponge over the enclosed sampling area (10 cm × 10 cm) for a total of approximately 10 times in the vertical and 10 times in the horizontal directions. The pressure for swabbing would be as if you were removing dried blood from the carcass. However, the pressure should not be too hard as to crumble or destroy the sponge.

Note: The template may need to be ''rolled'' from side to side during swabbing since the surface of the carcass is not flat. This ensures that the 100 cm^2 area is enclosed while swabbing.

17. After swabbing the belly area, transfer the template to the same hand that is holding the sponge. Do not contaminate the sponge or the inner edges of the sampling area of the template.

18. Climb the ladder or platform, holding onto the handrail with the hand used to hold the sampling template in place. Once at a convenient and safe height for sampling the ham, transfer template back to the ''climbing'' hand (hand used to hold onto the rail while climbing the ladder), taking care not to contaminate the sponge or the inner edges of the template.

19. Repeat steps 14–16 for the ham sampling area, using the SAME surface of the sponge used to swab the belly area.

20. After swabbing the ham area, carefully place the template back to the same hand that is holding the sponge. Do not contaminate the sponge or the inner edges of the sampling area of the template.

21. While holding the handrail, climb down from the ladder.

22. Transfer the template back to the ''climbing'' hand (hand used to hold onto the rail while descending the ladder), taking care not to contaminate the sponge or the inner edges of the template.

23. Repeat steps 14–16 for the jowl area, using the ''clean'' surface or side (the side that was not previously used to swab the belly/ham areas).

24. After swabbing the jowl area, carefully place the sponge back into the sponge bag. Do not touch the surface of the sponge to the outside of the sponge bag.

25. Add the additional BPD (about 15 ml) to the bag to bring the total volume to approximately 25 ml.

26. Press wire closures of the sponge bag together, expel excess air, then fold down the top edge of the bag 3 or 4 times. Secure the bag by folding the attached wire tie back against the bag. Place the closed sponge bag into the second bag and close the second bag securely.

27. (a) If samples are to be analyzed at an ON-SITE LABORATORY, begin sample preparation (ANALYTICAL METHODS section).

(b) If samples are to be analyzed at an OUTSIDE (OFF-SITE) LABORATORY, follow procedure in the Sample Shipment section.

Sample Shipment

Samples analyzed on-site must be analyzed as soon after collection as possible. If no on-site facilities are available, the samples must be shipped the same calendar day as collected, to

an outside laboratory. The samples must be analyzed no later than the day after collection.

1. Prechill shipping container by placing the open shipping container in the refrigerator at least the day before sampling.

2. Place the appropriately-labeled, double-bagged sample(s) in the prechilled shipping container in an upright position to prevent spillage. Newspaper may be used for cushioning the sample and holding it in the upright position. If more than one sample is collected during the day, take steps to ensure that samples are maintained at refrigeration temperature. Refrigeration temperatures help limit multiplication of any microorganisms present which ensures the most accurate results.

3. Place a corrugated cardboard pad on top of samples. This corrugated cardboard pad prevents direct contact of frozen gel packs with the samples. Next place the frozen gel pack(s) on top of the corrugated pad. Use sufficient frozen coolant to keep the sample refrigerated during shipment to the designated laboratory. Insert foam plug and press it down to minimize shipper head space.

4. Ship samples (via overnight delivery or courier) to the assigned laboratory.

Analytical Methods

Samples must be analyzed using one of the *E. coli* (Biotype I) quantitation methods found in the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC), International, 16th edition, or by any method which is validated by a scientific body in collaborative trials against the three tube Most Probable Number (MPN) method and agreeing with the 95% upper and lower confidence limits of the appropriate MPN index.

Suggested Quantitation Schemes

If a generic one ml plating technique is used for *E. coli* quantitation for cattle or swine carcass sponging sample analysis, the plate count would be divided by 12 to equal the count per cm^2. To cover the marginal and unacceptable range for *E. coli* levels (described in later section), the undiluted sample extract, a 1:10, a 1:100, a 1:1,000 and a 1:10,000 dilution should be plated, preferably in duplicate. Higher or lower dilutions may need to be plated based on the specific product.

If a hydrophobic grid membrane filtration method were used, the only difference would be filtration of one ml of the undiluted sample extract, 1:10, 1:100, 1:1,000 and 1:10,000 dilutions.

Additional dilutions of the original extract may need to be used if a three tube MPN protocol is used. The three highest dilutions that were positive for *E. coli* are used to calculate the MPN. MPN values from the appropriate MPN Table represent the count per ml of original extract and therefore must be divided by 12 to obtain the count per cm^2 of carcass surface area.

Record Keeping

Each test result must by recorded in terms of colony forming units per square centimeter (cfu/cm^2). A process control table or chart can be used to record the results and facilitate evaluation. Results should be recorded in the order of sample collection and include information useful for determining appropriate corrective actions when problems occur. The information needed for each sample includes date and time of sample collection, and, if more than one slaughter line exists, the slaughter line from which the sample was collected. These records are to be maintained at the establishment for twelve months and must be made available to Inspection Program employees on request. Inspection personnel review results over time, to verify effective and consistent process control.

For *E. coli* testing to be the most useful for verifying process control, timeliness is important and the record should be updated with the receipt of each new result. Detailed records should also be kept of any corrective actions taken if process control deviations are detected through microbiological testing.

Applying Performance Criteria to Test Results

Categorizing Test Results

E. coli test levels have been separated into 3 categories for the purpose of process control verification: acceptable, marginal, and unacceptable. (In the Pathogen Reduction/HACCP Regulation, the upper limits for the acceptable and marginal ranges were denoted by m and M.) These categories are described by slaughter species in Table 3.

TABLE 3.—VALUES FOR MARGINAL AND UNACCEPTABLE RESULTS FOR E. COLI PERFORMANCE CRITERIA

Slaughter class	Acceptable range	Marginal range	Unacceptable range
Cattle	Negative*	Positive but not above 100 cfu/cm^2	Above 100 cfu/cm^2.
Swine	10 cfu/cm^2	Above 10 cfu/cm^2 but not above 10,000 cfu/cm^2.	Above 10,000 cfu/cm^2.

*It should be noted that negative here is defined by the sensitivity of the sampling and test method used in the Baseline survey (5 cfu/cm^2 carcass surface area).

To illustrate the use of Table 3, consider a steer/heifer slaughter establishment. E. *coli* test results for this establishment will be acceptable if negative, marginal if positive but not above 100 cfu/cm^2, and unacceptable if above 100 cfu/cm^2.

Verification Criteria

The verification criteria are applied to test results in the order that samples are collected. The criteria consist of limits on occurrences of marginal and unacceptable results.

As each new test result is obtained, the verification criteria are applied anew to evaluate the status of process control with respect to fecal contamination.

1. An unacceptable result should trigger immediate action to review process controls, discover the cause if possible, and prevent recurrence.

2. A total of more than three marginal or unacceptable results in the last 13 consecutive results also signals a need to review process controls.

This way of looking at the number of marginal and unacceptable results is described as a ''moving window'' approach in the regulation. With this approach, results are accumulated until 13 have been accrued. After this, only the most recent 13 results—those in the ''moving window''—are considered.

An example of a record of results for Steer/Heifer testing is shown (in table form) below for an establishment performing two tests per day.

Test #	Date	Time collected	Test result (cfu/cm^2)	Result unacceptable?	Result marginal?	Number marginal or unacceptable in last 13	Pass/fail?
1	10–07	08:50	10	No	Yes	1	Pass
2	.....	14:00	Negative	No	No	1	Pass
3	10–08	07:10	50	No	Yes	2	Pass
4	.....	13:00	Negative	No	No	2	Pass
5	10–09	10:00	Negative	No	No	2	Pass
6	.....	12:20	Negative	No	No	2	Pass
7	10–10	09:20	80	No	Yes	3	Pass
8	.....	13:30	Negative	No	No	3	Pass
9	10–11	10:50	Negative	No	No	3	Pass
10	.....	14:50	Negative	No	No	3	Pass
11	10–14	08:40	50	No	Yes	4	Fail
12	.....	12:00	Nonegative	No	No	4	Fail
13	10–15	09:30	Negative	No	No	4	Fail
14	.....	15:20	Negative	No	No	3	Pass
15	10–16	07:30	Negative	No	No	3	Pass
16	.....	11:40	Negative	No	No	2	Pass
17	10–17	10:20	120	Yes	No	3	Fail

The following observations can be made on this example:

1. As of 10–14 at 08:40, there are four marginal or unacceptable results in the last 11 results, which exceeds the limit of 3 in 13 consecutive tests.

2. The limit of 3 in 13 also is exceeded for the next two tests, but since no new marginal or unacceptable result has occurred, these failures should not be treated as evidence of a new problem. The log or documentation of corrective action taken for the first failure should be adequate to verify that the deviation or problem was addressed.

3. On 10–15 at 15:20 the number of marginal or unacceptable results in the last 13 tests goes down to 3 because the marginal result for 10–07 at 08:50 is dropped and replaced by an acceptable result as the 13-test window moves ahead 1 test.

4. The result for 10–17 at 10:20 exceeds 100 and is unacceptable.

Figure 4 shows the same results as the above example but the results are displayed in chart form. The numbers along the horizontal axis of the graph (x-axis), refers to the test number in the chart above. The information for each test result, such as the time and date the sample was collected could also be recorded on the chart.

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Figure 1. Example of sampling template (not drawn to scale)

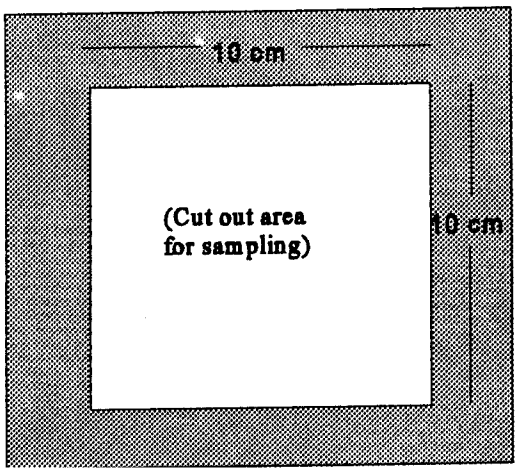
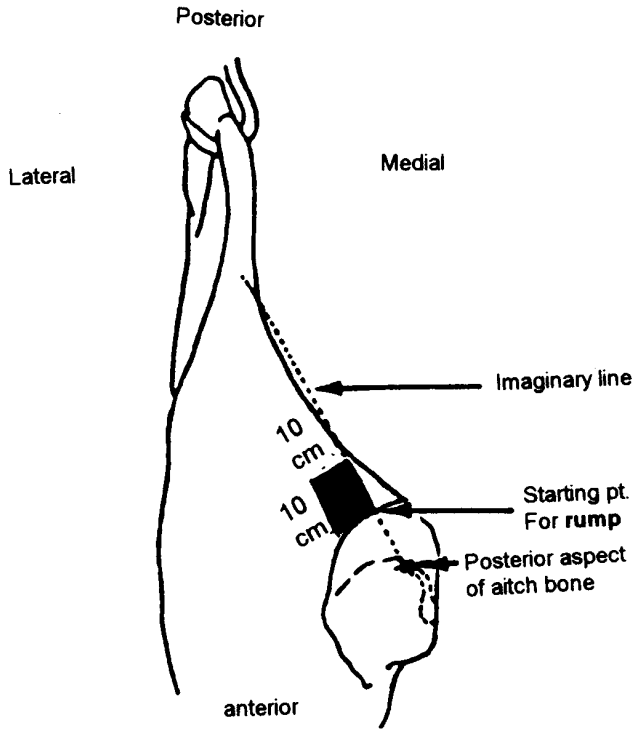


Figure 2. Sampling locations for *E. coli* testing of cattle carcasses

Rump Locate the posterior aspect of the aitch bone. Draw an imaginary line toward the achilles tendon. At the point where the line intersects the cut surface of the round is the starting point for the rump sample. Measure 10 cm up the line leading to the achilles tendon, then 10 cm over (laterally), then 10 cm back to the cut surface of the round, then 10 cm along the cut surface to form the 10 cm by 10 cm square area.

Note: The upper illustration has been purposely altered somewhat. A true lateral view of the carcass would not show the aitch bone. From a medial view, the whole 10 cm x 10 cm sampling area could not be seen. Therefore, a lateral view with a portion of the round removed so the location of the aitch bone is shown is illustrated.



Flank Locate the cutaneous flank muscle (external abdominal oblique) and follow the medial border of the muscle anteriorly until it comes with approximately 3" of the midline. This will be the starting point. Measure up (posteriorly) 10 cm (approximately 4 inches) along a line approximately 3" from the midline (measure up or parallel to the midline), then over (laterally) 10 cm (approximately 4 inches) to form a 10 cm wide by 10 cm long square sample.

Brisket Locate the elbow of the carcass. Draw an imaginary line straight across (medially) to the midline cut. This will be the starting point. Measure up along the midline 10 cm (approximately 4 inches), then over 10 cm (approximately 4 inches) to form a 10 cm wide by 10 cm long square sample.

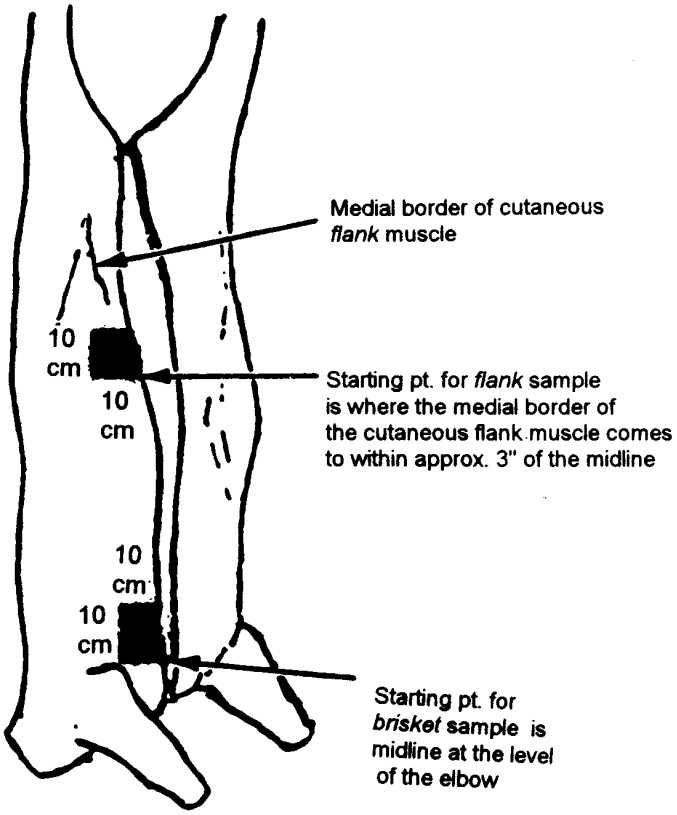
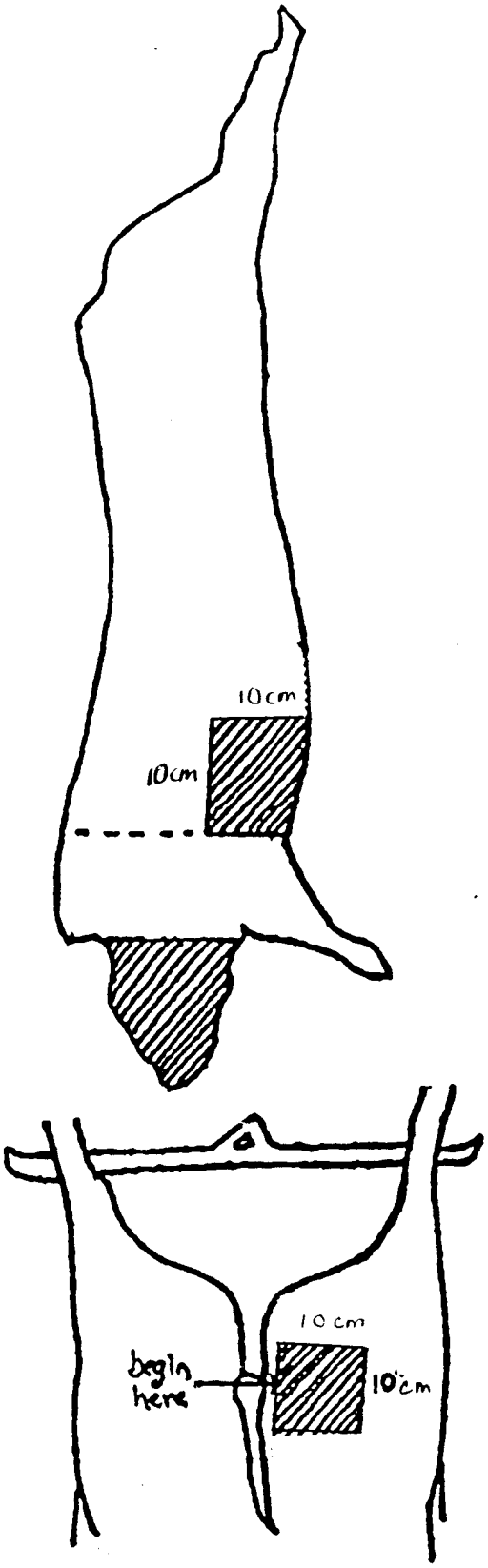


Figure 3. Sampling locations for *E. coli* testing of swine carcasses



belly Locate the elbow of the carcass. Draw an imaginary line straight across (medially) to the midline cut. This will be the starting point. Measure up along the midline 10 cm (approximately 4 inches), then over 10 cm (approximately 4 inches) to complete the 10 cm long by 10 cm wide square sample. This square area will be the 100 cm^2 area to swab for the belly sample.

jowls Draw an imaginary line from the atlas/axis joint to the ventral midline; all skin below that point will be considered the jowl.

ham From the dorsal position, locate the lateral surface of the base of the tail, and measure up (caudal) 5 cm along the lateral edge of the exposed fat margin, then 10 cm laterally. Now measure 10 cm down (cranial), then 10 cm medially, then 5 cm up (posteriorly) to complete a 10 cm long by 6 cm wide rectangular sampling area.

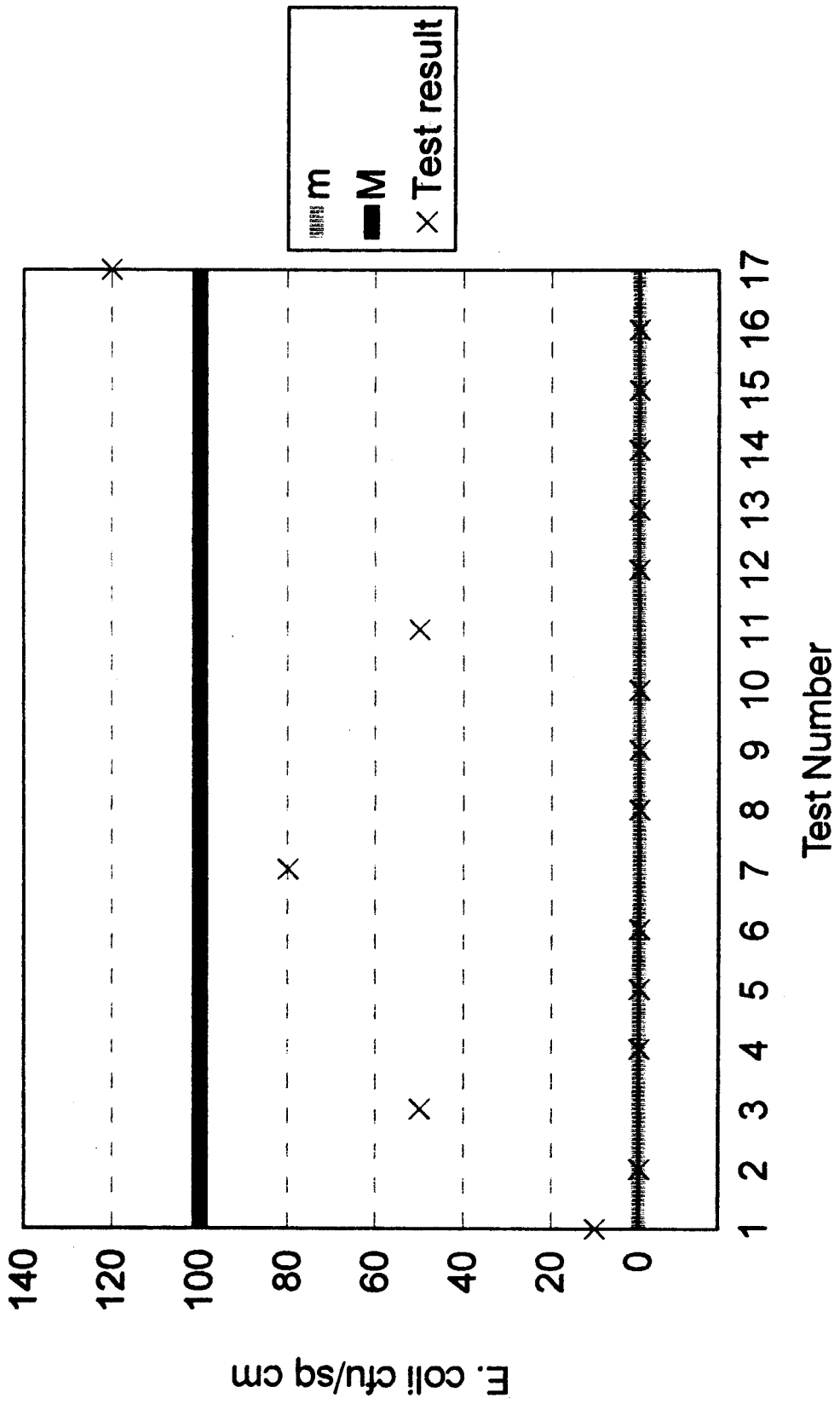


Figure 4. Example of *E. coli* results using a control chart

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Appendix G—Guidelines for Escherichia coli Testing for Process Control Verification in Poultry Slaughter Establishments

Introduction

Under the Pathogen Reduction/HACCP Regulation, all poultry slaughter establishments will be required to test carcasses for generic *E. coli* as a tool to verify process control. This document outlines the sampling and microbial testing that should be followed to meet this requirement. It also gives guidance to interpreting your results. This document is a supplement to the Regulation, but not a substitute for it. Further in-depth details of the program may be found in the Regulation. Please provide these guidelines to your company microbiologist or testing laboratory in order to help you meet the regulatory requirements for generic *E. coli* testing.

Guidelines for Sample Collectors/Microbiologists

Background

This sampling protocol has been prepared to support the Pathogen Reduction/HACCP Regulation. Carcass sampling for broiler and turkey carcasses remain the nondestructive whole bird rinse which was used in the FSIS Nationwide Microbiological Baseline Data Collection Programs.

Carcasses within the same establishment and in different establishments must be sampled and analyzed in the same manner if the results are to provide a useful measure of process control across the nation. It is imperative that all like establishments adhere to the same sampling and analysis requirements detailed here, without deviation. These sampling and analytical procedures may be directly written into your establishment's individual HACCP plan.

Poultry carcasses must be sampled after the chill tank at the end of the drip line or last readily accessible point prior to packing/cut-up. This sample collection location is the same as that in the FSIS baseline studies, making samples taken here comparable to the nationwide baseline performance criteria.

Pre-sampling Preparation

Sample collection will be carried out by the individual designated in the establishment's written protocol for microbiological sampling. The protocol should include a check list of tasks to be performed prior to sample collection, materials needed for sample collection, random selection procedures, where the samples will be analyzed (on-site versus off-site), and other information that will aid the sample collector. As stated previously, this guideline can be a part of the plant's sample collection guidelines, but plant specific details and procedures will need to be included. Sampling supplies, such as sterile gloves, sterile sampling solutions, hand soap, sanitizing solution, etc., need to be assembled prior to beginning sample collection.

Sterile sampling solutions, Butterfield's phosphate diluent (BPD), can be stored at room temperature. However, at least on the day prior to sample collection, check solutions for cloudiness (DO NOT use solutions that are cloudy, turbid or contain particulate matter) and place the number of containers of sampling solution (BPD) that will be needed for the next day's sampling in the refrigerator.

To obtain the most accurate results, samples should be analyzed as soon after collection as possible. However, if samples must be transported to an off-site laboratory, the samples need to be maintained at refrigeration temperatures until transport, then shipped refrigerated via an overnight delivery service to the laboratory performing the analysis. Samples analyzed off-site must be picked up by the overnight courier the SAME calendar day the sample is collected. The sample must arrive at the laboratory no later than the day after the sample is collected. Samples shipped to an outside laboratory must be analyzed no later than the day after collection. The following section gives information on shipping containers and transporting samples to off-site facilities.

Shipping Containers and Coolant Packs

It is important that samples fit easily into the shipping containers so that the sample bags do not break.

Correct use of the refrigerant gel-ice packs and proper packing of the shipping container are necessary so that samples arrive at the laboratory at an acceptable temperature. Frozen samples or samples which are too warm are not considered valid and must not be analyzed. Some bacteria may be damaged by temperatures that are too cold, while temperatures that are too warm can allow bacteria to reproduce. Maintaining samples at improper temperatures may cause inaccurate sample results.

The sample should be kept refrigerated, NOT FROZEN, in the shipping container prior to pickup by the courier service. The shipping container, itself, should not be used as a refrigerator. However, multiple samples (if needed) for that day may be stored in the open shipping container in the cooler or refrigerator.

Sampling Frequency

Sampling frequency for *E. coli* testing is determined by production volume. The required minimum testing frequencies for all but very low production volume establishments are shown in Table 1 by slaughter species.

TABLE 1.—E. COLI TESTING FREQUENCIES [a]

Chickens	1 test per 22,000 carcasses.
Turkeys	1 test per 3,000 carcasses.

[a] **Note:** These testing frequencies do not apply to very low volume establishments. See Table 2.

Very Low Volume Establishments

Some establishments may be classified as very low volume establishments based on their annual production volume. The maximum yearly slaughter volumes for very low volume establishments are described in Table 2.

TABLE 2.—MAXIMUM YEARLY POULTRY SLAUGHTER VOLUMES FOR VERY LOW VOLUME ESTABLISHMENTS

Slaughter species	Criteria (yearly slaughter volume)
Chickens	Not more than 440,000 birds.
Turkeys	Not more than 60,000 birds.
Chickens and turkeys.	Not more than 440,000 total, with not more than 60,000 turkeys.

Establishments with very low volumes are to sample the predominant species once per week, initially, until at least 13 test results have been obtained.

Once the initial criteria have been met for very low volume establishments (see APPLYING PERFORMANCE CRITERIA TO TEST RESULTS), the establishment will repeat the same sampling regime once per year, in the 3 month period of June through August, or whenever a change is made in the slaughter process or personnel.

Random Selection of Carcasses

Samples are to be taken randomly at the required frequency (See section on Sampling Frequency). For example, given the frequency of testing for turkeys is 1 (one) test per every 3,000 turkeys slaughtered, then if a plant slaughters 1,500 turkeys an hour, 1 (one) sample will be taken every 2 hours.

Different methods of selecting the specific carcass for sampling could be used, but all require the use of random

numbers. Methods could include: using random number tables, using calculator- or computer-generated random numbers, drawing cards, etc. When selecting the random numbers, use the method(s) currently in use at the establishment for other sampling programs, if other programs are currently underway.

The carcass for sampling must be selected at random from all eligible carcasses. If multiple lines exist, randomly select the line for sample collection for that interval. Repeat the random selection process for the next sampling interval. Each line should have an equal chance of being selected at each sampling interval.

Poultry Carcass Selection

The poultry carcasses will be selected at random after chilling, at the end of the drip line or last readily accessible point prior to packing/cut-up. A WHOLE carcass is required, that is, one that has not been trimmed.

Note: If more than one shift is operating at the plant, the sample can be taken on any shift, provided the following requirements are met:

Selection of TIME: Select the time, based on the appropriate sampling frequency, for collecting the sample.

Selection of CHILLER: If more than one chiller system is in operation at the time of sample collection, the chill tank from which the sample is selected must be randomly selected.

Selection of POULTRY CARCASS: Based on the frequency of sampling for your establishment, identify a carcass (selected by your random number method) from the predetermined point, and then count back five (5) carcasses and select the next carcass for sampling. Exception: If the fifth carcass is not a WHOLE (untrimmed) bird, count back an additional five carcasses for sample selection. Each carcass must have an equal chance of being selected. The reason for counting back five carcasses is to avoid any possible bias during selection.

Aseptic Techniques/Sampling

Extraneous organisms from the environment, hands, clothing, sample containers, sampling devices, etc., may lead to erroneous analytical results. Stringent requirements for microbiological analysis are necessary, therefore, use of aseptic sampling techniques and clean sanitized equipment and supplies are of utmost importance.

There should be an area designated for preparing sampling supplies, etc. A stainless steel, wheeled cart or table would be useful during sampling. A small tote or caddy could be easily moved to the location of sampling and could be used for carrying supplies, supporting sample bags when adding sterile solutions to sample bags, etc.

Sterile gloves should be used for collecting samples. The only item which may contact the external surface of the glove is the exposed sample being collected. Keep in mind that the outside surfaces of the sample container are not sterile. Do not handle the inside surface of the sterile sample containers. Do not touch anything else. The following procedure for putting on sterile gloves can be followed when collecting samples:

(a) Peel open the package of sterile gloves from the top without contaminating (touching, breathing on, contacting, etc.) the exterior of the gloves.

(b) Remove a glove by holding it from the wrist-side opening inner surface. Avoid any contact with the outer surface of the glove. Insert the washed and sanitized hand into the glove, taking care not to puncture the glove.

(c) Next, taking care not to contaminate the outer surface of the glove, repeat the step above for the hand you will use to physically handle the sample.

(d) If at any time you are concerned that a glove may be contaminated, discard it and begin again with Step (a) above.

Preparation for Sample Collection

Prior to collecting samples, review appropriate sampling steps, random selection procedures, and other information that will aid in sample collection.

On the day prior to sample collection, after checking for cloudiness/turbidity, place the number of Butterfield's phosphate diluent (BPD) containers that will be needed for the next day's sampling in the refrigerator/cooler. If samples will be shipped to an off-site facility, pre-chill shipping container and refrigerator packs (follow manufacturer's directions for gel-packs).

On the day of sampling, gather all sample collection bags, sterile gloves, sanitizer, hand soap, sterile solutions for sampling (BPD), and specific materials listed under the *Materials* section of the sample collection section for the type of carcass to be sampled. Ensure that all sampling supplies are on hand and readily available before beginning sample collection.

Label the sample bags before starting the sampling procedure. Use permanent ink. If you are using paper labels, it is important that the label be applied to the bag at normal room temperature; it will not stick if applied in the cooler.

Outer clothing (frocks, gloves, head gear, etc.) worn in other areas of the plant should be removed before entering the sampling area or preparing to collect samples. Replace outer clothing removed earlier with clean garments (i.e., laboratory coat) that have not been directly exposed to areas of the plant outside of the sampling area.

Sanitize the sample work area surfaces by wiping with a clean disposable cloth or paper towel dipped in a freshly prepared 500 ppm sodium hypochlorite solution (0.05% sodium hypochlorite) or other approved sanitizer which provides an equivalent available chlorine concentration. The sample work area surfaces must be free of standing liquid before sample supplies and/or product containers are placed on them.

Before sampling, thoroughly wash and scrub hands to the mid-forearm. Use antibacterial hand soap. If available, this should include a sanitizer at 50 ppm equivalence available chlorine. Dry the hands using disposable paper towels.

Specific Sample Collection Procedures

Chicken Carcass Rinse Sampling Procedure

Materials

1. 2 Sterile 3500 milliliter (ml) stomacher-type or ziplock-type bags or equivalent. (The bag must be sterile and should be large enough to hold the carcass while rinsing.)

2. 400 ml sterile, Butterfield's phosphate diluent (BPD).

3. Plastic tie wraps or equivalent (if needed to secure the bag).

4. Sterile gloves.

5. Optional—(See alternate sampling—step 10)—Sterile leak-proof container.

Collection

Read the sections under Pre-sampling Preparation and Preparation for Sample Collection before beginning the sampling procedure. Use the predetermined random selection procedure to select the carcass to sample. The randomly selected bird will be collected after the chiller, at the end of the drip line as follows:

1. Ensure all sampling supplies are present and have been properly labeled. An assistant may be helpful during sampling.

2. Open a large stomacher-type bag without touching the sterile interior of the bag. (Rubbing the top edges of the bag between the thumb and forefinger will cause the opening to gap for easy opening.)

3. Put on sterile gloves.

4. With one hand, push up through the bottom of the sampling bag to form

a ''glove'' over one hand with which to grab the bird, while using your other hand to pull the bag back over the hand that will grab the bird. This should be done aseptically without touching the exposed interior of the bag.

5. Using the hand with the bag reversed over it, pick up the bird by the legs (hocks) through the stomacher bag. (The bag functions as a 'glove' for grabbing the bird's legs.) Take care not to contaminate the exposed interior of the bag. Allow any excess fluid to drain before reversing the bag back over the bird. (Alternately, have an assistant hold open the bag. Using your gloved hand, pick up the bird by the legs, allow any fluid to drain, and place the bird in the sampling bag.)

6. Rest the bottom of the bag on a flat surface. While still holding the top of the bag slightly open, add the sterile BPD (400 ml) to the bag containing the carcass, pouring the solution over the carcass.

(Alternately, with the aid of an assistant holding the bag open, add the sterile BPD (400 ml) to the bag containing the carcass, pouring the solution over the carcass.)

7. Expel most of the air from the bag, then close the top of the bag. While securely holding the bag, rinse the bird inside and out using a rocking motion for 30 shakes (approximately one minute). This is done by holding the bird through the bottom of the bag with one hand and the closed top of the bag with the other hand. Hold the bird securely and rock it in an arcing motion, alternating the weight of the bird from one hand to the other (motion like drawing an invisible rainbow or arch), assuring that all surfaces (interior and exterior of the carcass) are rinsed.

8. Rest the bag with the bird on a flat surface and, while still supporting the bird, open the bag.

9. With a gloved hand, remove the carcass from the bag. Since the carcass was rinsed with a sterile solution, it can be returned to the chill tank. Be sure not to touch the interior of the bag with your gloved hand.

10. Secure the top of the bag so that the rinse fluid will not spill out or become contaminated.

(Alternately, at least 30 milliliters of rinse fluid can be poured into a sterile leak-proof container to be sent to the lab for analysis.)

11. Place the sample bag (or leak-proof container) into another bag and secure the opening of the outer bag.

12. (a) If samples are to be analyzed at an ON-SITE LABORATORY, begin sample preparation for the selected method of analysis.

(b) If samples are to be analyzed at an OUTSIDE (OFF-SITE) LABORATORY, follow the procedure in the Sample Shipment section.

Turkey Carcass Rinse Sampling Procedure

Materials

1. 2 Sterile 3500 ml stomacher-type or ziplock-type bags or equivalent. (The bag must be sterile and should be large enough to hold the carcass while rinsing, the bags FSIS will be using for the Salmonella sampling program measure approximately 18″ × 24″. Large turkeys should be placed in a plain, clear polypropylene autoclave bag , about 24″ × 30″ to 36″).

2. 600 ml sterile, Butterfield's phosphate diluent (BPD)

3. Plastic tie wraps or thick rubber bands or equivalent, if needed to secure sample bag

4. Sterile gloves

5. Optional—sterile, leak-proof container (see step 12 Alternate procedure)

Collection

Read the sections under Pre-sampling Preparation and Preparation for Sample Collection before beginning the sampling procedure. Use a predetermined random selection procedure to select the carcass to be sampled. The randomly selected bird will be collected after the chiller, at the end of the drip line as follows:

1. Ensure that all supplies are on hand and readily available. An assistant will be needed to hold the bag for collecting the bird.

2. Have an assistant open the large sterile stomacher-type bag (designated for rinsing the carcass) and be ready to receive the turkey carcass. (Rubbing the top edges of the bag between the thumb and index finger will cause the opening to gap open).

(Alternately: If no assistant is available, place the closed large sampling bag into a bucket or pail (e.g., use the bag to ''line'' a bucket like a trash-can liner), then open the bag. The bucket will be used as a holder or stand to support the bag. Do not contaminate the inner surfaces of the sampling bag.)

3. Put on sterile gloves.

4. Remove the selected turkey from the drip line by grasping it by the legs and allowing any fluid to drain from the cavity.

5. Place the turkey carcass, vent side up, into a sterile sampling bag. Only the carcass should come in contact with the inside of the bag.

6. Manipulate the loose neck skin on the carcass through the bag and position it over the neck bone area to act as a cushion and prevent puncturing of the bag. The assistant will need to support the carcass with one hand on the bottom of the bag.

7. While still supporting the bottom of the bag, have the assistant open the bag with the other hand. Alternately, rest the bottom of the bag on a pre-sanitized surface (i.e. a table), and while still supporting the carcass in the bag, open the bag with the other hand.

8. Add the sterile BPD (600 ml) to the bag containing the carcass, pouring the diluent over the carcass.

9. Take the bag from the assistant and expel excess air from the bag and close the top. While securely holding the bag, rinse the bird inside and out using a rocking motion for 30 shakes (approximately one minute). This is done by holding the carcass through the bag with one hand and the closed top of the bag with the other hand. Holding the bird securely with both hands, rock in an arcing motion alternating the weight of the bird from one hand to the other (motion like drawing an invisible rainbow or arch), assuring that all surfaces (interior and exterior of the carcass) are rinsed.

10. Hand the bag back to the assistant.

11. With a gloved hand, remove the carcass from the bag letting excess fluid drain back into the bag. Since the carcass was rinsed with a sterile solution, it can be returned to the chill tank. Be sure not to touch the interior of the bag with your gloved hand.

12. Expel excess air, taking care not to expel any rinse fluid. Secure the top of the bag so that the rinse fluid will not spill out or become contaminated.

(Alternately, *at least* 30 milliliters of rinse fluid can be poured into a sterile, leak-proof container and sent to the lab for analysis.)

13. Place the sample bag (or container) into another bag and secure the opening of the outer bag.

14. (a) If samples are to be analyzed at an ON-SITE LABORATORY, begin sample preparation for the selected method of analysis. (See Analytical Methods section.)

(b) If samples are to be analyzed at an OUTSIDE (OFF-SITE) LABORATORY, follow the procedure in the Sample Shipment section.

Sample Shipment

Samples analyzed on-site must be analyzed as soon after collection as possible. If no on-site facilities are available, the samples must be shipped the same calendar day as collected, to an outside laboratory. The samples must be analyzed no later than the day after collection.

1. Prechill shipping container by placing the open shipping container in the refrigerator at least the day before sampling.

2. Place the appropriately-labeled, double-bagged sample in the prechilled shipping container in an upright position to prevent spillage. Newspaper may be used for cushioning the sample and holding it in the upright position. Ensure that samples are maintained at refrigeration temperature. Refrigeration temperatures limit multiplication of any microorganisms present.

3. Place a corrugated cardboard pad on top of samples. The corrugated pad prevents direct contact of frozen gel packs with the samples. Next, place the frozen gel pack(s) on top of the corrugated pad. Use sufficient frozen coolant to keep the sample refrigerated during shipment to the designated laboratory. Insert foam plug and press it down to minimize shipper head space.

4. Ship samples (via overnight delivery or courier) to the assigned laboratory.

Analytical Methods

Samples must be analyzed using one of the *E. coli* (Biotype I) quantitation methods found in the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC), International, 16th edition, or by any method which is validated by a scientific body in collaborative trials against the three tube Most Probable Number (MPN) method and agreeing with the 95% upper and lower confidence limits of the appropriate MPN index.

Suggested Quantitation Schemes

For poultry rinse fluid samples, if a generic one ml plating technique is used for *E. coli* quantitation, the plate count would not have to be divided to get the count per ml of rinse fluid. To cover the marginal and unacceptable range for *E. coli* levels (described in later section), the undiluted extract (optional), a 1:10, a 1:100, a 1:1,000 and a 1:10,000 dilution should be plated, preferably in duplicate. Higher or lower dilutions may need to be plated based on the specific product.

If a hydrophobic grid membrane filtration method were used, the only difference would be filtration of one ml of the undiluted extract (optional), 1:10, 1:100, 1:1,000 and 1:10,000 dilutions.

Additional dilutions of the original extract may need to be used if a three tube MPN protocol is used. The three highest dilutions that were positive for *E. coli* are used to calculate the MPN.

Record Keeping

Results of each test must by recorded, in terms of colony forming units per milliliter rinse fluid (cfu/ml) for chicken and turkeys. A process control table or chart can be used to record the results and facilitate evaluation. Results should be recorded in the order of sample collection and include information useful for determining appropriate corrective actions when problems occur. The information needed for each sample includes date and time of sample collection, and, if more than one slaughter line exists, the slaughter line from which the sample was collected. These records are to be maintained at the establishment for twelve months and must be made available to Inspection Program employees on request. Inspection personnel review results over time, to verify effective and consistent process control.

For *E. coli* testing to be the most useful for verifying process control, timeliness is important and the record should be updated with the receipt of each new result. Detailed records should also be kept of any corrective actions taken if process control deviations are detected through microbiological testing.

Applying Performance Criteria to Test Results

Categorizing Test Results

E. coli test levels have been separated into 3 categories for the purpose of process control verification: acceptable, marginal, and unacceptable. (In the Pathogen Reduction/HACCP Regulation, the upper limits for the acceptable and marginal ranges were denoted by m and M.) These categories are described by slaughter species in Table 3.

TABLE 3.—VALUES FOR MARGINAL AND UNACCEPTABLE RESULTS FOR E. COLI PERFORMANCE CRITERIA

Slaughter class	Acceptable range	Marginal range	Unacceptable range
Chicken	100 cfu/ml or less	Over 100 cfu/ml but not over 1,000 cfu/ml	Above 1,000 cfu/ml.
Turkey	NA*	NA*	NA*.

*The FSIS Baseline study has not been completed for this slaughter class. Levels will be set upon completion of this baseline.

To illustrate the use of Table 3, consider a chicken slaughter establishment. *E. coli* test results for this establishment will be acceptable if not above 100 cfu/ml, marginal if above 100 cfu/ml but not above 1,000 cfu/ml, and unacceptable if above 1,000 cfu/ml.

Verification Criteria

The verification criteria are applied to test results in the order that samples are collected. The criteria consist of limits on occurrences of marginal and unacceptable results.

As each new test result is obtained, the verification criteria are applied anew to evaluate the status of process control with respect to fecal contamination.

1. An unacceptable result should trigger immediate action to review process controls, discover the cause if possible, and prevent recurrence.

2. A total of more than three marginal or unacceptable results in the last 13 consecutive results also signals a need to review process controls.

This way of looking at the number of marginal and unacceptable results is described as a ''moving window'' approach in the regulation. With this approach, results are accumulated until 13 have been accrued. After this, only the most recent 13 results—those in the ''moving window''—are considered.

An example of a record of results for Chicken testing is shown (in table form) below for an establishment performing two tests per day.

Test No.	Date	Time collected	Test result (cfu/ml)	Result unacceptable?	Result marginal?	Number marginal or unacceptable in last 13	Pass/ Fail?
1	10–07	08:50	120	No	Yes	1	Pass.
2		14:00	10	No	No	1	Pass.
3	10–08	07:10	150	No	Yes	2	Pass.
4		13:00	50	No	No	2	Pass.
5	10–09	10:00	([1])	No	No	2	Pass.
6		12:20	10	No	No	2	Pass.
7	10–10	09:20	800	No	Yes	3	Pass.
8		13:30	10	No	No	3	Pass.
9	10–11	10:50	10	No	No	3	Pass.
10		14:50	10	No	No	3	Pass.
11	10–14	08:40	500	No	Yes	4	Fail.
12		12:00	30	No	No	4	Fail.
13	10–15	09:30	10	No	No	4	Fail.
14		15:20	10	No	No	3	Pass.
15	10–16	07:30	10	No	No	3	Pass.
16		11:40	10	No	No	3	Pass.
17	10–17	10:20	1,200	Yes	No	3	Fail.

[1] Negative.

The following observations can be made on this example:

1. As of 10–14 at 08:40, there are four marginal or unacceptable results in the last 11 results, which exceeds the limit of 3 in 13 consecutive tests.

2. The limit of 3 in 13 also is exceeded for the next two tests, but since no new marginal or unacceptable result has occurred, these failures should not be treated as evidence of a new problem. The log or documentation of corrective action taken for the first failure should be adequate to verify that the deviation or problem, if any, was addressed.

3. On 10–15 at 15:20 the number of marginal or unacceptable results in the last 13 tests goes down to 3 because the marginal result for 10–07 at 08:50 is dropped replaced by an acceptable result as the 13-test window moves ahead 1 test.

4. The result for 10–17 at 10:20 exceeds 1,000 and is unacceptable.

The Figure 1 shows the same results as above displayed in chart form. The numbers along the horizontal axis of the graph (x-axis) refer to the test number in the chart above. The information for each test result, such as the time and date the sample was collected could also be recorded on the chart.

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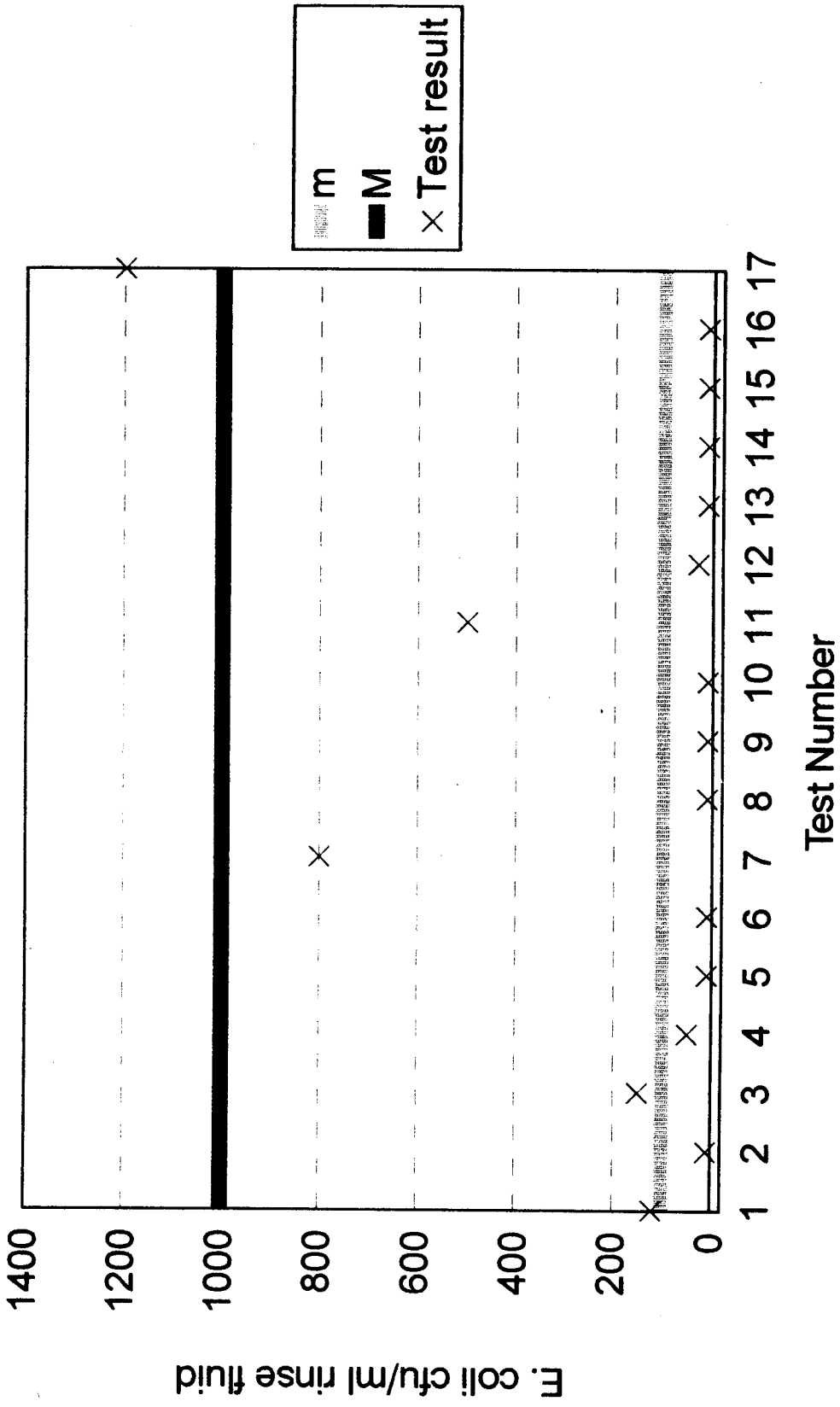

Figure 1. Example of *E. coli* results using a control chart

BILLING CODE 3410–DM–C

Note: The following Supplement will not appear in the Code of Federal Regulations.

Supplement—Final Regulatory Impact Assessment for Docket No. 93–016F, ''Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems.''

Table of Contents

Appendix A to Final Regulatory Impact Assessment

I. Introduction

A. Purpose

In docket No. 93–016F, the Food Safety and Inspection Service (FSIS) is promulgating new regulations that require an estimated 9,079 inspected meat and poultry establishments to adopt a Hazard Analysis and Critical Control Points (HACCP) processing control system covering all production operations within 3½ years of final rule publication. The regulation also requires that all 9,079 establishments adopt and implement standard operating procedures (SOP's) for sanitation and establishes, for the first time, food safety performance standards for microorganisms on raw meat and poultry products. This final rule establishes pathogen reduction performance standards for *Salmonella* that are established using the current pathogen prevalence as determined by the national baseline studies. These standards are not directed at judging whether specific lots of a product are adulterated under the law. Rather, compliance with the standards will be determined by a statistical evaluation of the prevalence of bacteria in each establishment's products. FSIS will implement sampling programs to determine compliance with the *Salmonella* standard. The rule does not require inspected establishments to test for *Salmonella*. The pathogen reduction performance standards apply to 2,682 slaughter establishments and another estimated 2,840 establishments that produce raw ground product but do not have slaughter operations.

The final rule also requires that all slaughter establishments test for generic *E. coli* to verify process control for fecal contamination during slaughter and sanitary dressing. Results will be measured against performance criteria established from the national baseline surveys. Under this final rule, the 2,682 inspected slaughter establishments will be required to verify by microbial testing that they are controlling their slaughter and sanitary dressing processes in accordance with the performance criteria. The rule establishes testing frequencies based on production levels, but does not establish the performance criteria as enforceable regulatory standards. As the preamble points out, the criteria will be flexible and subject to change as FSIS and the industry gain experience with them and accumulate more data on establishment performance. The criteria are intended specifically to provide an initial basis upon which slaughter establishments and FSIS can begin to use microbial testing to evaluate the adequacy of establishment controls for slaughter and sanitary dressing procedures.

The objective of this regulation is to reduce the risk of foodborne illness from meat and poultry. The focus is on reducing and eventually minimizing the risk from the following four pathogens:

- *Campylobacter jejuni/coli.*
- *Escherichia coli O157:H7.*
- *Listeria monocytogenes.*
- *Salmonella.*

This document is the final Regulatory Impact Analysis (RIA) prepared in compliance with the provisions of Executive Order 12866 and analyses requirements of the Regulatory Flexibility Act (P.L. 96–354) and the Unfunded Mandates Reform Act (P.L. 104–4). The purpose of this final RIA is to evaluate alternatives to and costs and benefits associated with a mandatory HACCP-based regulatory program for all meat and poultry establishments under inspection.

B. Methodology

The methodology used to develop cost estimates for this final RIA is relatively straightforward. The costs estimates are based on data for average wages, the cost of specific processing equipment or the cost of conducting specific laboratory analyses.

The benefits analysis is less straightforward. The analysis has defined regulatory effectiveness as the percentage of pathogens eliminated at the manufacturing stage. The benefits analysis concludes that there is insufficient knowledge to predict with certainty the effectiveness of the proposed rule. Without specific predictions of effectiveness, FSIS has calculated projected health benefits for a range of effectiveness levels.

The link between regulatory effectiveness and health benefits is the assumption that a reduction in pathogens leads to a proportional reduction in foodborne illness. FSIS has presented the proportional reduction calculation as a mathematical expression that facilitates the calculation of a quantified benefit estimate for the purposes of this final RIA. FSIS has not viewed proportional reduction as a risk model that would have important underlying assumptions that merit discussion or explanation. For a mathematical expression to be a risk model, it must have some basis or credence in the scientific community. That is not the case here. FSIS has acknowledged that very little is known about the relationship between pathogen levels at the manufacturing

stage and dose, i.e., the level of pathogens consumed.

There are many factors that play important roles in the actual link between pathogen levels at the manufacturing stage and frequency of foodborne illness. First, the effectiveness definition of ''percentage of pathogens reduced'' can refer to the percentage of packages that contain pathogens or the level of pathogens within packages. The pathogens-to-illness relationship is further complicated because cross-contamination in kitchens is believed to play a major role. It can not be assumed that a reduction in the number of pathogens present in a package of meat or poultry will prevent a cross-contamination related illness. On the other hand, given that the number of consumed pathogens necessary to cause illness (threshold) can be different for every possible pathogen or individual combination, a reduction in pathogen levels at the time of packaging may prevent illness for many cross-contamination scenarios.

These types of unknowns illustrate why the relationship between pathogen levels and foodborne illness levels remains unknown. As stated above, without a known relationship, FSIS has used the proportional reduction assumption to provide a quantified estimate, recognizing that the real relationship is probably different for each pathogen and category of meat and poultry product.

Risk minimization as the objective of this rule means the elimination of most foodborne illness caused by the contamination of meat and poultry products in inspected establishments by any of the four pathogens listed above. The reduction in pathogens needed to do this is unknown and would vary for individual pathogens and products.

This final RIA includes a discussion of the status of risk assessment for foodborne pathogens that responds to the new Departmental guidelines for preparing risk assessments contained in Departmental Regulation 1521–1, December 21, 1995. Although the statutory requirements for risk analysis included in the Federal Crop Insurance Reform and Department of Agriculture Reorganization Act of 1994 (P.L. 103–354) do not apply to this final rule, there were public comments on the need for additional risk assessment or risk analysis. This final RIA includes the Agency's response to those comments.

On February 3, 1995, FSIS published a preliminary RIA as part of the proposed Pathogen Reduction HACCP rule (60 F.R. 6871). The preliminary RIA announced the availability of a detailed supplemental cost analysis, titled ''Costs of Controlling Pathogenic Organisms on Meat and Poultry,'' which was available from the FSIS Docket Clerk during the comment period. This final RIA will refer to the analysis published with the proposed rule and the supplemental cost analysis collectively as the ''preliminary analysis.''

During the public comment period the Department conducted a number of public hearings, technical conferences and information briefings. On May 22, 1995, the Agency conducted a special hearing in Kansas City dealing with the impacts of the proposed rule on small businesses. In July 1995, FSIS conducted a survey of the State inspection programs to collect additional information to assess the impact on State establishments.

This final RIA is based on the preliminary RIA, the supplemental cost analysis, all written public comments, the records from public hearings including the meeting on small business impacts, the survey of State programs, and any new information or data that have become available during the comment period. The analysis also refers specifically to cost estimates developed by the Research Triangle Institute (RTI) during personal interviews with nine establishments that previously participated in the FSIS HACCP Pilot Program. The RTI report, *HACCP Pilot Program Cost Findings,* August 31, 1994, which was referred to in both written and public hearing comments were developed under contract to FSIS in 1994.

C. Summary Comparison of Costs and Benefits—Proposal to Final

FSIS estimated that the proposed rule would have 20-year industry costs of $2.2 billion. Those costs are presented in Table 1, organized by the regulatory components identified in the proposal.

The estimated costs for the final rule are also presented in Table 1. For some of the regulatory components, it is easy to track the costs from the proposal to the final rule. For example, the costs for Sanitation SOP's remain essentially the same. The reduction from $175.9 to $171.9 million reflects the change in implementation period from 90 days to six months.

The costs for developing and implementing HACCP plans are also directly comparable. The estimated cost has increased for the HACCP component of plan development. FSIS has increased its estimate for this cost after reviewing the public comments and assessing the overall impact on plan development costs of the decisions to eliminate the requirements for implementing time/temperature and antimicrobial treatment requirements prior to HACCP implementation. In the preliminary analysis, the cost for developing HACCP plans was reduced because of the experience that establishments would have gained in developing their plans for implementing time/temperature and antimicrobial treatment requirements.

TABLE 1.—COMPARISON OF COSTS—PROPOSAL TO FINAL

[$ Millions—Present Value of 20-year Costs]

Regulatory component	Proposal	Final
I. Sanitation SOP's	175.9[a]	171.9
II. Time/Temperature Requirements	45.5	0.0
III. Antimicrobial Treatments	51.7	0.0
IV. Micro Testing	1,396.3[b]	174.1
V.		
Compliance with *Salmonella* standards	Not Separately Estimated[c]	55.5–243.5
Compliance with generic *E. coli* criteria	Not Applicable	Not Separately Estimated
VI. HACCP:		
Plan Development	35.7	54.8
Annual Plan Reassessment	0.0	8.9
Recordkeeping (Recording, Reviewing and Storing Data)	456.4	440.5[d]
Initial Training	24.2	22.7[d]
Recurring Training	0.0	22.1[e]
VII. Additional Overtime	20.9	17.5[d]

TABLE 1.—COMPARISON OF COSTS—PROPOSAL TO FINAL—Continued

[$ Millions—Present Value of 20-year Costs]

Regulatory component	Proposal	Final
Subtotal—Industry Costs	2,206.6	968.0–1,156.0
VIII. FSIS Costs	28.6[f]	56.5
Total	2,235.2	1,024.5–1,212.5

[a] The preliminary analysis included a higher cost estimate for sanitation SOP's ($267.8 million) that resulted because of a programming error. The cost estimate of $175.9 million is based on an effective date of 90 days after publication.

[b] The preliminary analysis was based on the premise that microbial testing would be expanded to cover all meat and poultry processing after HACCP implementation. The proposed rule only required sampling for carcasses and raw ground product. Thus, the cost estimate of $1,396.3 million was higher than the actual cost of the proposed sampling requirements.

[c] The preliminary analysis accounted for some of the cost of complying with the new standards under the regulatory components of micro testing, antimicrobial treatments, and time and temperature requirements.

[d] These costs are slightly different from the proposal because of changes in the implementation schedule.

[e] FSIS added costs for recurring training based on the review of public comments.

[f] Based on current estimates for the cost of training, inspector upgrades, and $0.5 million for annual HACCP verification testing.

Table 1 shows that FSIS has added two categories of HACCP costs that were not included in the preliminary cost analysis. A cost for recurring annual HACCP training was added in response to comments that there would be recurring costs because of employee turnover. FSIS also added a minimal cost for annual reassessment of HACCP plans, although the Agency believes that reassessment will be negligible for establishments successfully operating under a HACCP plan.

Table 1 shows that the proposed requirements for time and temperature specifications and antimicrobial treatments have not been included in the final rule. The preliminary analysis treated these items as interim costs that were incurred prior to HACCP implementation. For the time and temperature requirements, the preliminary analysis identified both one-time capital equipment costs and recurring recordkeeping costs. The time and temperature recordkeeping costs were assumed to become part of the HACCP recordkeeping costs. The recurring costs for antimicrobials were assumed to end with HACCP implementation. The preliminary analysis indicated that at the time of HACCP implementation, the slaughter establishments would make a decision on whether to continue the antimicrobial treatments and employ other methods to reduce the microbial load on carcasses. The preliminary analysis did not, however, include a cost component for either continuing the antimicrobial treatments or adding alternative pathogen reduction methods.

Under the micro testing component, the final rule requires that all 2,682 slaughter establishments implement microbial sampling programs using generic *E. coli*. The 20-year cost of this requirement is $174.1 million. After HACCP implementation including validation that the *E. coli* performance criteria are being met, establishments may use alternate testing programs unless FSIS specifically objects. In addition, in the period prior to mandatory HACCP, FSIS will consider exemptions on a case-by-case basis for establishments that are currently using an alternative *E. coli* sampling frequency if the establishment can provide data demonstrating the adequacy of its existing program. The cost estimate of $174.1 million assumes that all slaughter establishments continue to test at the frequencies outlined in the final rule.

Up to this point, all the costs discussed have been predictable in the sense that they refer to a specific requirement directing all establishments or a specific category of establishments to take a well-defined action. FSIS has developed point estimates for all predictable costs. In contrast, the pathogen reduction performance standards for *Salmonella* do not prescribe a set of actions that establishments must take. Because the standards are set using the national prevalence estimates from the baseline studies, the Agency is also not able to predict how many establishments are already meeting the standards or how many will have to modify their current operations to comply.

The cost analysis in Section V recognizes that the performance standards create a set of potential costs for 5,522 establishments, 2,682 slaughter establishments and another estimated 2,840 establishments that produce raw ground product but do not have slaughter operations. The analysis estimates potential costs by developing two scenarios that lead to a range of possible costs depending on how the different industry sectors will respond to the new standards and depending on how many establishments will need to modify their production processes in order to comply.

Reducing pathogens for slaughter establishments involves either modifying the incoming animals or birds, improving the dressing procedures so as to reduce contamination during procedures such as hide removal and evisceration, or using interventions such as antimicrobial treatments to kill or remove the pathogens following contamination. For many establishments, the process of implementing HACCP programs may, by itself, improve the dressing procedures sufficiently to meet the new standards. Other establishments may have to choose between slowing production lines, modifying some attribute of their incoming live animals or birds, or adding post-dressing interventions such as the new steam vacuum process or antimicrobial rinses.

The 2,840 raw ground processing operations will have to control their incoming ingredients either by conducting their own testing or by requiring that suppliers meet purchase specifications. The cost analysis also recognizes that even though the rule does not require the 2,682 slaughter establishments to test for *Salmonella*, some establishments may conduct their own *Salmonella* testing programs to avoid failing a series of tests conducted by the Agency. Thus, it can be argued that the Agency's intent to implement establishment specific testing for *Salmonella* is indirectly requiring the industry to routinely monitor their *Salmonella* levels to assure they will be in compliance.

As shown in Table 1, the two scenarios developed in the cost analysis lead to a range in cost estimates of $55.5 to $243.5 million to comply with the new pathogen reduction standards. Some of these costs are contained in the

Table 1 proposal costs of $51.7 for antimicrobial treatments and the $1,396.3 for micro testing that included the cost of having 5,522 establishments conduct daily *Salmonella* testing for each species slaughtered and each variety of raw ground product produced.

The two cost scenarios were developed to illustrate potential costs for compliance with standards established using the current pathogen prevalence as determined by the national baseline studies. These standards move the Agency's regulatory program in the direction of meeting the food safety objective of minimizing the risk of foodborne illness from pathogens that contaminate meat and poultry products. The Agency has stated its intent to establish tighter standards over time. The Agency recognizes that future tighter standards could impose a new set of compliance costs. To illustrate, where the use of hot water rinses may be adequate to assure compliance with the *Salmonella* standards as established for this rule, such rinses may not be adequate to assure compliance with future standards. Any change in the standards will, however, be implemented through additional rulemaking. At that time the Agency will have extensive data on the distribution of pathogens by establishment and better data on the cost and effectiveness of different interventions. These data enhancements will allow for improved cost analysis of future standard setting activities. Inspected establishments need to consider the Agency's overall food safety objectives when making decisions on capital investments designed to assure compliance with the food safety standards established by this rulemaking.

The cost analysis in Section V also recognizes that the performance criteria for generic *E. coli* create a set of potential costs for 2,682 slaughter establishments. A line for these costs is shown in Table 1 along with the entry that these costs were not separately quantified.

As discussed in Section V, the anticipated actions to comply with the generic *E. coli* criteria are the same as the anticipated actions to comply with the standards for *Salmonella*. FSIS has concluded that if the low cost scenario for *Salmonella* compliance proves to be more accurate, then the Agency would expect to see some compliance costs for the generic *E. coli* performance criteria. If the high cost scenario is correct, then the compliance actions taken to assure compliance with the *Salmonella* standards should also assure compliance with the generic *E. coli* criteria.

Finally, Table 1 includes a cost of $17.5 million associated with additional overtime charges for inspection. While it is recognized that final decisions on the future of the Agency's Total Quality Control (TQC) program have not been made, this analysis includes a conservative impact assumption that the existing TQC regulations will be withdrawn.

Both the preliminary and final analysis identify a maximum potential 20-year public health benefit from $7.13 to $26.59 billion that is tied to eliminating establishment-related contamination from four pathogens on meat and poultry. The contamination from these four pathogens at the manufacturing stage leads to an estimated annual cost of foodborne illness ranging from $0.99 billion to $3.69 billion. The maximum 20-year benefit results from eliminating this annual cost of foodborne illness beginning in the fifth year after publication. Although there is reason to believe significant benefits will be generated during the first four years, for analytical purposes FSIS used the conservative estimate that benefits do not begin until all establishments have HACCP systems in place and pathogen reduction standards for *Salmonella* apply to all establishments that slaughter or produce raw ground product.

There are two principle reasons why benefits will begin to accrue before the fifth year. First, the HACCP requirements and *Salmonella* standards apply to large establishments at 18 months and small establishments at 30 months. The large slaughter establishments account for over 74 percent of total carcass weight. Second, the generic *E. coli* testing requirements are effective six months after publication. The generic *E. coli* results will provide both establishment management and inspection program personnel a tool by which to assess establishments' control over slaughter and sanitary dressing procedures. Although the generic *E. coli* criteria are not being established as regulatory standards, FSIS believes their use will lead to improved control over slaughter and sanitary dressing procedures which will, in turn, lead to reductions in fecal contamination and corresponding reductions in contamination by enteric pathogens. Rather than attempt to estimate the benefits associated with reduced contamination resulting from use of generic *E. coli* testing, this analysis has assumed public health benefits begin in the fifth year. By that time all establishments have had an opportunity to adjust their *E. coli* sampling programs based on their HACCP programs.

The low and high estimates for potential benefits are due to the current uncertainty in estimates for incidence of foodborne illness and death. If the low potential benefit estimate is correct, the analysis shows that the new HACCP-based program must reduce pathogens by 15 to 17 percent for benefits to outweigh projected costs. If the high estimate is the correct estimate, the new program needs to reduce pathogens by only 4 to 5 percent to generate net societal benefits.

As discussed in Section III, there are other benefits to this rule that have not been quantified. Examples include increased public protection from physical hazards and the increased production efficiency that accompanies improved process control.

In the preliminary analysis FSIS took the position that quantified pathogen reduction benefits were related to the overall proposed HACCP-based regulatory program and that there was no way to distribute benefits among the five different components that made up the proposed rule. Under the proposed rule it was essentially impossible to determine the proportion of pathogen reduction benefits that could be attributable to the proposed pathogen reduction standards versus the proposed antimicrobial treatments or time-temperature requirements or the proposed mandatory HACCP programs. Given the revised structure of the final rule, this analysis attributes pathogen reduction benefits to the requirements that all establishments implement HACCP systems and that if those systems are implemented in slaughter establishments or establishments shipping raw ground product, they must have critical limits set to assure compliance with the new pathogen reduction standards for *Salmonella*. However, as discussed above, FSIS believes that pathogen reduction benefits will begin to occur when establishments start using the generic *E. coli* results to assess their control over slaughter and sanitary dressing procedures.

FSIS believes that the Sanitation SOP's component of this final rule has significant benefits in terms of increased productivity for inspection resources. The HACCP component also has productivity benefits in addition to public health benefits. One of the reasons FSIS has not yet achieved a program that can focus appropriate resources on the risks of microbial pathogens is that in recent years

national budget problems have provided limited increases in Agency resources compared to the increase in its responsibilities generated by industry growth, the Federal takeover of more State programs, and new food production technologies and products. For most of its history, the inspection program was able to obtain additional resources when it took on new responsibilities. Now FSIS is faced with taking on new responsibilities with the same resources.

The final rule is a necessary component of an FSIS management strategy that will raise the productivity of current resources so that the program can maintain all its consumer protection objectives. Raising productivity requires raising outputs, reducing inputs or any combination of the two that gets more done for less. Productivity can be increased in today's inspection program by: (1) focusing resource use on the basis of risk, giving the highest priority to safety objectives; (2) clarifying the respective responsibilities of government and industry to assure the best use of government resources; and (3) designing new methods of inspection that are more efficient than existing inspection but which maintain or improve consumer protection.

The Sanitation SOP's and HACCP requirements are designed to accomplish objectives in all three of the above areas. With SOP's FSIS can monitor sanitation plans with fewer resources than it takes to conduct comprehensive sanitation reviews. The benefit of the SOP's is, therefore, the capacity to reallocate inspection resources to other activities where the payoff in terms of reducing the risk of foodborne illness may be greater. With SOP's there is less likelihood that establishments will be able to substitute the inspector's sanitation review for their own sanitation program. Similarly, with HACCP there is less likelihood that firms can use inspection as a substitute for their own control programs. In both cases productivity is enhanced by clarifying responsibilities. The benefits associated with increased productivity are difficult to quantify because the precise reallocation of inspection resources is not yet clear.

Finally, with the implementation of this rule, FSIS intends to introduce new methods of inspection that are more efficient than those currently in place. As noted above, more efficient methods is the third way in which productivity can be increased in the inspection system.

II. Regulatory Alternatives

A. Market Failure

Consumers make choices about the food they purchase based upon factors such as price, appearance, convenience, texture, smell, and perceived quality. In an ideal world, people would be able to make these decisions with full information about product attributes and choose those foods which maximize their satisfaction. In the real world, however, information deficits about food safety complicate consumer buying decisions.

Since all raw meat and poultry products contain microorganisms that may include pathogens, raw food unavoidably entails some risk of pathogen exposure and foodborne illness to consumers. However, the presence and level of this risk cannot be determined by a consumer, since pathogens are not visible to the naked eye. Although they may detect unwholesomeness from obvious indications such as unpleasant odor or discoloration caused by spoilage microorganisms, consumers cannot assume products are safe in the absence of spoilage. They simply have no clear-cut way to determine whether the food they buy is safe to handle and eat.

When foodborne illness does occur, consumers often cannot correlate the symptoms they experience with a specific food because some pathogens do not cause illness until several days, weeks or even months after exposure. Thus, food safety attributes are often not apparent to consumers either before purchase or immediately after consumption of the food. This information deficit also applies to wholesalers and retailers who generally use the same sensory tests—sight and smell—to determine whether a food is safe to sell or serve.

The societal impact of this food safety information deficit is a lack of accountability for foodborne illnesses caused by preventable pathogenic microorganisms. Consumers often cannot trace a transitory illness to any particular food or even be certain it was caused by food. Thus, food retailers and restaurateurs are generally not held accountable by their customers for selling pathogen-contaminated products and they, in turn, do not hold their wholesale suppliers accountable.

This lack of information applies equally to small businesses. Some small businesses have argued for exemption from the rule because they sell most of their product to family, friends and neighbors, but they are overlooking the fact that perhaps the majority of foodborne illness victims may believe they had some type of flu virus or other illness and have no idea that their illness was foodborne and, if they do, they have no idea as to the source. Without feedback, (i.e., without a connection of product to illness), there is no market where buyers and sellers have sufficient information upon which to judge purchase decisions. Without feedback there is insufficient incentive to make substantial improvements in process control.

This lack of marketplace accountability for foodborne illness means that meat and poultry producers and processors have little incentive to incur extra costs for more than minimal pathogen controls. The widespread lack of information about pathogen sources means that businesses at every level from farm to final sale can market unsafe products and not suffer legal consequences or a reduced demand for their product. An additional complication is that raw product is often fungible at early stages of the marketing chain. For example, beef from several slaughterhouses may be combined in a batch of hamburger delivered to a fast food chain. Painstaking investigation by public health officials in cases of widespread disease often fails to identity foodborne illness causes; in half the outbreaks the etiology is unknown.

Most markets in industrialized economies operate without close regulation of production processes in spite of consumers having limited technical or scientific knowledge about goods in commerce. Branded products and producer reputations often substitute for technical or scientific information and result in repeat purchases. Thus, brand names and product reputations become valuable capital for producers.

In the U.S. food industry, nationally recognized brand names have historically provided significant motivation for manufacturers to ensure safe products. In recent years, more and more raw meat and poultry have come to be marketed under brand names. Nevertheless, not even all brand name producers produce their products under the best available safety controls. Further, a significant part of meat and poultry, particularly raw products, are not brand name products and are not produced under conditions that assure the lowest practical risk of pathogens.

The failure of meat and poultry industry manufacturers to produce products with the lowest risk of pathogens and other hazards cannot be attributed to a lack of knowledge or appropriate technologies. The science and technology required to significantly

reduce meat and poultry pathogens and other hazards is well established, readily available and commercially practical.

Explanations for why a large portion of the meat and poultry industry has not taken full advantage of available science and technology to effectively control manufacturing processes include the following:

1. Meat and poultry processing businesses are relatively easy to enter; there are no training or certification requirements for establishment operators. Consequently, the level of scientific and technical knowledge of management in many establishments is minimal.

2. The industry is very competitive and largely composed of small and medium-sized firms that have limited capital and small profits.

3. Management in many of these establishments has little incentive to make capital improvements for product safety because results from that investment are not distinguishable by customers and therefore yield no income.

In spite of these barriers, many industry establishments do produce meat or poultry products using process controls that assure the lowest practical risk of pathogens and other hazards.

FSIS has concluded that the lack of consumer information about meat and poultry product safety and the absence of adequate incentives for industry to provide more than minimal levels of processing safety represents a market failure requiring Federal regulatory intervention to protect public health.

B. General Regulatory Approaches

The problem of microbial pathogens in meat and poultry has become increasingly apparent. Documented cases of foodborne illness each year, some of which have resulted in death, represent a public health risk that FSIS judges to be unacceptable. Within existing authorities there are four broad regulatory approaches the Department could use to address this unacceptable public health risk.

- Market Incentives.
- Information and Education.
- Voluntary Industry Standards.
- Government Standards.

The final rule represents the fourth approach.

The above discussion on market failure summarizes why FSIS has concluded that the market will not address the public health risk resulting from microbial pathogens in meat and poultry.

The role and effectiveness of consumer and food service worker education in assuring food safety was raised in public comments. For example, comments suggested that since most foodborne illness involves temperature abuse or consumer/food handler mishandling, consumer education offers the most cost-effective approach. FSIS sees a clear role for education and agrees that education is essential for assuring food safety. However, experience has shown that education alone has limited effectiveness in reducing foodborne illness. The effectiveness of education for food safety, and, indeed, for improving diets and other food related behavior, has not been demonstrated. FSIS views education as a valuable adjunct to other regulatory approaches, but it has no evidence that a major increase in education expenditures will produce the behaviors required to reduce foodborne illness.

A voluntary industry standard would call for the formation of a standards setting group, such as the American National Standards Institute (ANSI) to develop and publish a voluntary standard. Compliance with such a voluntary standard would be determined by third-party testing and certification. For example, Underwriter's Laboratory (UL) tests and certifies electronic components for industry-wide standards. FSIS has not seen any evidence that the industry is prepared to undertake, or even desires a voluntary standards approach. This is understandable. Because the principles underlying the safe production of meat and poultry are the same regardless of who administers the standards, an industry administered system is likely to be more expensive and less effective than a government one. The lack of power to mandate participation reduces the value of standard setting to participants, since foodborne illness episodes attributable to non-participants tend to raise suspicion of all similar products. Further, the industry would be called upon to pay the enforcement cost which under the present rule would be paid by the government.

For these reasons, the Department concludes that mandatory process control regulations offer the best approach for addressing this unacceptable public health risk.

C. Need For Improved Process Control

FSIS has determined that effective process control is needed throughout the meat and poultry industry in order to minimize pathogen contamination and control other health hazards. Accordingly, a regulatory strategy has been formulated to mandate process control improvements to achieve immediate reductions and an eventual minimization of the risk of meat and poultry pathogens, chemical, and physical hazards in the nation's food supply. This strategy is supported by consumers, scientists, and the majority of meat and poultry industry processors who already recognize the benefits of good process control.

Process control is a proactive strategy that all segments of industry can undertake to anticipate manufacturing problems in advance and prevent unsafe foods from being produced. In practice, process control is a systematic means to:

- Identify and control production hazards.
- Determine control points in the processing system.
- Establish standard measures for each control point.
- Set procedures for establishment workers to monitor requirements.
- Provide clear instructions for appropriate corrective actions when a control point goes out of control.
- Establish record-keeping to document control point measurements.
- Provide procedures for verification tests to ensure that the system continues to operate as planned.

The process control strategy summarized in this paper is founded on three principles:

1. USDA regulatory policy should be focused on providing a solution to meat and poultry biological, chemical, and physical hazards that present the highest public health risks.

2. It is essential that the Nation's food safety system address pathogenic microorganisms which present the greatest foodborne risk to human health.

3. These pathogens and resulting risks of foodborne illness can be largely avoided by uniform meat and poultry industry efforts to attain and maintain more effective methods of control during the manufacturing process.

The focus of this strategy is explicitly on prevention; it is designed to prevent the production of defective product as opposed to more costly and less effective detect-and-condemn methods.

Process control is not a substitute for inspection any more than inspection could be a substitute for process control. This distinction is important because Federal inspection was never intended to be—and cannot be—the front-line control for food safety in meat and poultry processing establishments. Safety controls must be built into the manufacturing process and be administered continuously by industry. The objective of inspection in a process control environment is to assure that those controls are present, adequate, and properly used.

To summarize, the process control regulatory strategy promulgated by this rule will among its other well established attributes, correct two important deficiencies in the nation's current food safety effort. It will: (1) provide industry the tools and incentive to reduce meat and poultry pathogens as a means to improve food safety, and (2) help focus Federal inspection on the highest product, process and establishment risks, and, at the same time, clarify that the industry is responsible for producing safe meat and poultry, while the Government's role is oversight.

Factors Considered in Evaluating a Process Control Strategy

The process control regulatory strategy was evaluated using five factors for effectiveness. A processing control program is effective if it:

1. Controls production safety hazards.
2. Reduces foodborne illness.
3. Makes inspection more effective.
4. Increases consumer confidence.
5. Provides the opportunity for increased productivity.

The following sections discuss these five effectiveness factors that have been applied to evaluate process control alternatives.

Controls Production Safety Hazards

Process control is a system for identifying food hazards and reducing or eliminating the risks they present. In operation, control points are established in a food production line where potential health hazards exist; management of these points has proven to be effective in reducing the probability that unsafe product will be produced. Ongoing records of each process control will enable establishment managers and quality control personnel to spot trends that could lead to problems and devise a strategy that prevents them before they occur.

Detection by end product testing is not a viable alternative to process control because it only sorts good product from bad and does not address the root cause of unacceptable foods. Additionally, keeping ''bad'' foods out of commerce through sorting end product is possible only when tests and standards for sampling are well established and it is practical only where the ''test'' is not expensive because sorting requires a huge number of samples for reliability.

Reduces Foodborne Illness

As industry improves its control over the safety aspects of meat and poultry production, foodborne illness will begin to decline. This is the principal non-negotiable goal for both USDA and industry.

The precise occurrence of human health problems attributed to pathogenic microorganisms or other potential foodborne hazards, such as chemical contaminants, animal drug residues, pesticides, extraneous materials, or other physical contaminants is not known. Foodborne illness is nevertheless recognized by both domestic and international scientists as a significant public health problem and there is wide agreement that pathogenic microorganisms are the major cause of food-related disease. The estimated annual (not discounted) cost of foodborne illness attributable to meat and poultry products from the four pathogens that are the focus of this regulation is from $1.1 to $4.1 billion. FSIS estimates that 90 percent of this annual cost, $0.99 to $3.69 billion, is attributable to contamination that occurs in establishments.

Makes Inspection More Effective

Currently, the FSIS inspectors in meat and poultry establishments that are not assigned to slaughter line positions perform selected inspection tasks that generate independent data about an establishment's production processes and environment. This activity produces ''snapshots'' of establishment operations at a particular moment. In contrast, process control generates records of establishment performance over time. These records and periodic verification inspections will enable FSIS inspectors to see how an establishment operates at all times, i.e., whether and where processing problems have occurred, and how problems were addressed.

The availability of more and better processing data will establish trends that set benchmarks from which deviations can be more quickly and accurately assessed. USDA inspectors will be trained to spot these deviations and take action when needed to ensure establishments bring a faulty process back into control. This type of Federal oversight is substantially more effective than a regulatory program that merely detects and condemns faulty end products. In the words of the National Advisory Committee on Microbiological Criteria for Foods, ''Controlling, monitoring, and verifying processing systems are more effective than relying upon end-product testing to assure a safe product.''

Increases Consumer Confidence

The number of foodborne illness outbreaks and incidents attributable to pathogens in meat or poultry raise questions about whether Federal inspection is as effective as it should be. Highly visible public controversies about meat and poultry inspection indicate an erosion of public confidence in the safety of meat and poultry products. There are growing demands that USDA improve its regulation of pathogens. The process control regulatory strategy described in this paper is USDA's response to those demands.

Many outbreaks of foodborne illness have been determined to be caused by mishandling of meat and poultry products after federally inspected processing. USDA believes that additional efforts to reduce pathogens during manufacturing will reduce these risks as well. This coupled with the improved retail regulatory controls from state adoption and enforcement of the Food Code should reduce this cause of illness. The Food Code is an FDA publication, a reference that provides guidance to retail outlets such as restaurants and grocery stores and institutions such as nursing homes on how to prepare food to prevent foodborne illness. State and local regulatory bodies use the FDA Food Code as a model to help develop or update their food safety rules and to be consistent with national food regulatory policy.

A significant portion of the meat and poultry industry do not take advantage of readily available methods to control their manufacturing processes. The Department has concluded that further regulation will bring industry standards up to what can practically be achieved in the manufacture of meat and poultry products through current scientific knowledge and available process control techniques. Raising the safety floor through regulations that mandate better process control will demonstrate to the public that USDA and industry are making a concerted effort to reduce the risk of foodborne illness from meat and poultry.

The economic benefits of increased consumer confidence can be conceptually realized as the amount consumers would be willing to pay for safer food. This ''willingness to pay'' reflects consumer desires to avoid foodborne illness and the expected medical and other costs associated with it. However, the data are not available to make quantitative estimates of this benefit.

Provides the Opportunity for Increased Productivity

Better process control is a sound and rational investment in the future of our

nation's meat and poultry industry. USDA's process control strategy will educate industry management about the need and methodology for development of a consistent, preventive, problem-solving approach to safety hazards, which can be expanded to other business objectives such as product quality and production efficiency. There is considerable evidence of how process control has improved worldwide industrial productivity in the past 40 years. This proposal will extend process control principles to parts of the meat and poultry industry that have not formerly used them.

Some important non-safety benefits that will accrue from industry use of better process control methods are:

- First, better production controls will result in more efficient processing operations overall with fewer product defects. Fewer defects mean less reworking, waste and give-away, resulting in increased yields and more profit opportunities.
- Second, better controls will significantly reduce the risk to processors that product with food safety defects will slip into commerce. Expensive and embarrassing product recalls can be, for the most part, avoided or greatly reduced with proper process controls.
- Third, better control of pathogens will impact all microorganisms, including those responsible for decomposition, resulting in quality improvement and longer shelf life for products.
- Fourth, better production controls improve establishment employee productivity which improves profit opportunities.

D. Regulatory Alternatives for Process Control

1. Mandatory HACCP

Considering the five effectiveness criteria of process control discussed above, the most effective means for generating the benefits reflected in these criteria is a mandatory HACCP regulatory program. This alternative clearly meets all five criteria described above. In fact, a mandatory HACCP program was judged to be the only option that will effect adequate processing improvements in all establishments throughout the industry. Only through mandatory HACCP can pathogen risks be minimized to the fullest extent possible; thereby significantly reducing foodborne illness, improving effectiveness of inspection, increasing consumer confidence, and ensuring a more viable industry. No other alternative accomplishes as much in these five areas as mandatory HACCP.

HACCP is a process control strategy that has been scientifically proven effective in food manufacturing establishments. HACCP is widely recognized by scientific authorities such as the National Academy of Sciences and international organizations such as the Codex Alimentarius. It is used today by a number of establishments in the food industry to produce consistently safe products. This approach has been supported for years by numerous groups that have studied USDA meat and poultry regulatory activities.

In 1983 FSIS asked the National Academy of Sciences (NAS) to evaluate the scientific basis of its inspection system and recommend a modernization agenda. The resulting report, ''Meat and Poultry Inspection, The Scientific Basis of the Nation's Program,'' National Academy Press, 1985 was the first comprehensive evaluation of a scientific basis for inspection. The 1985 NAS report provided a blueprint for change: it recommended that FSIS focus on pathogenic microorganisms and require that *all* official establishments operate under a HACCP system to control pathogens and other safety hazards.

After urging (NAS Recommendations, Page 4) the intensification of ''current efforts to control and eliminate contamination with micro-organisms that cause disease in humans,'' NAS encouraged (Page 135) USDA to ''move as vigorously as possible in the application of the HACCP concept to each and every step in establishment operations, in all types of enterprises involved in the production, processing, and storage of meat and poultry products.''

The General Accounting Office (GAO) has also identified needed improvements in USDA's present inspection system. In its reports and congressional testimony, and in numerous publications, GAO has endorsed HACCP as the most scientific system available to protect consumers from foodborne illness. This sentiment is most clearly expressed in a May 1994 report, ''Food Safety: Risk-Based Inspections and Microbial Monitoring Needed for Meat and Poultry,'' in which GAO recommended development of a mandatory HACCP program that includes microbial testing guidelines. GAO urged USDA to assist meat and poultry establishments in the development of their microbial testing programs by, among other things, disseminating information on the programs already in operation.

A third major proponent of HACCP is the National Advisory Committee on Microbiological Criteria for Foods (NACMCF), which was established in 1988 by the Secretary of Agriculture to advise and provide recommendations to the Secretaries of Agriculture and Health and Human Services on developing microbiological criteria to assess food safety and wholesomeness. Since 1989, NACMCF has prepared a series of reports on the development and implementation of HACCP. As one of its first tasks, the Committee developed ''HACCP Principles for Food Production'' in November 1989. In this report, the Committee endorsed HACCP as a rational approach to ensure food safety and set forth principles to standardize the technique. In 1992, the Committee issued an updated guide, ''Hazard Analysis and Critical Control Point System.''

In 1993 NACMCF defined the roles of regulatory agencies and industry in implementing HACCP. ''The Role of Regulatory Agencies and Industry in HACCP'' proposed responsibilities for FDA, USDA, and other agencies and industry during various phases of HACCP implementation. Similar suggestions for program change have been voiced by consumers, industry, state and local government representatives, as well as other constituent groups. For example, consumers at recent public hearings and the HACCP Round Table supported implementation of mandatory HACCP throughout the meat and poultry industry.

The meat and poultry industry has itself provided broad support for HACCP as a means to control pathogens, emphasizing that HACCP-based food production, distribution, and preparation can do more to protect public health than any Federal inspection program. They have recommended that HACCP be used to anticipate microbiological hazards in food systems and to identify risks in new and traditional products. State departments of health and agriculture have also endorsed the HACCP approach.

2. Alternatives to Mandatory HACCP

FSIS examined six other approaches before determining that mandatory HACCP was the most effective means for assuring process control in the meat and poultry industries.

1. Status quo
2. Intensify present inspection
3. Voluntary HACCP regulatory program
4. Mandatory HACCP regulation with exemption for small businesses
5. Mandatory HACCP regulation only for ready-to-eat products

6. Modified HACCP—recording deviations and responses only

These alternatives were assessed using the five effectiveness criteria presented in the previous section. The following six sections summarize the appraisal of each alternative.

Status Quo

This option would essentially continue establishment processing controls and Federal inspection as they are now. Good establishments with adequate methods for managing process lines would probably remain under control. The Agency, under its present authority, cannot shift resources out of good establishments so the situation of poor performing establishments is unlikely to change. This situation raises immediate questions about the first factor—controls production safety hazards—being met. Experience has proven that Federal inspection cannot substitute for management in establishments which have difficulty producing safe product consistently. Also, inspection cannot be as effective in the current establishment environment as in a process control establishment environment.

The status quo does not target industry and inspection resources on those hazards that lead to the greatest reduction in foodborne illness (factor two). In addition, food safety experts, consumers, and other observers have told USDA they are not satisfied with pathogen control by organoleptic methods as practiced in the present inspection program. Doing nothing new would perpetuate consumer doubts about the ability of Federal inspection to regulate pathogens which is counter to factor four. Consequently, the Department has concluded that business as usual is not an acceptable response to pathogens associated with meat and poultry products. Agency public health responsibilities alone require that more positive actions be taken.

Intensify Present Inspection

As one alternative to the proposed mandatory HACCP regulation, FSIS could intensify its present inspection system, i.e., focus new resources on suspected areas of risk in each establishment. This approach would assign to FSIS responsibility for designing, testing and mandating by specific regulation, process control systems for all meat and poultry products with potential safety hazards. A major flaw with this approach is that the burden of ensuring a safe product would be placed largely on FSIS instead of industry establishments where it belongs. Establishment management would have little motivation to become knowledgeable about process control or to implement process control systems.

The mandating of specific process controls has sometimes succeeded, as a regulatory strategy, for example, in correcting food safety problems in certain ready-to-eat products. However, these controls largely consisted of lethal heat treatments applied during final product processing. This approach is obviously inappropriate for product that is marketed raw which is most frequently associated with meat and poultry foodborne illness. The identification of processes that can be applied to raw product in every establishment would be much more difficult, if not impossible. Thus, intensified command-and-control regulation fails to meet the primary criterion for process control, i.e., control production safety hazards at all stages of meat and poultry slaughter and processing. Related to this failing, inspection would be ineffective without all establishments maintaining process control systems (factor three.) This option would not only require significant resource increases, it represents government taking on more, not less, responsibility for the production process, making it more difficult to focus on the highest risks of foodborne illness. With the burden of control and monitoring on USDA's inspection force rather than on establishment managers, industry performance in reducing foodborne illness would be unlikely to improve (factor two).

Voluntary HACCP Regulatory Program

A voluntary HACCP program would not provide reduction of pathogens uniformly across the processing spectrum because many in industry would choose not to participate. Therefore voluntary HACCP would not be sufficient to attain the necessary reduction in foodborne illness (factor two).

Voluntary HACCP would be implemented most frequently in establishments with good processing controls already, while establishments with unsophisticated controls would be less likely to participate. The explanation for this flaw is to be found in simple economics and, to a large degree, the attitudes of establishment management. Establishments with good processing controls now are most likely to adopt HACCP voluntarily because their management understands the linkage between how a product is handled during preparation and its finished quality and safety.

Conversely, establishments without good processing controls today are much less likely to participate in a voluntary HACCP program. These establishments are more often operated by management that lacks the knowledge or motivation to institute better processing controls. Nevertheless, it is precisely this group of low performing establishments that FSIS must reach to attain its public health goal. Nothing short of a mandatory HACCP regulatory program will be effective in bringing processing improvements to these marginal performers.

The Agency's regulation permitting the use of voluntary Total Quality Control (TQC) Systems provides a useful analogy to how effective a voluntary HACCP program would be. TQC focuses on establishment responsibility for meeting or exceeding the standards set by FSIS for all operations that are conducted in an establishment, including incoming raw materials, processing procedures, critical limits for product standards, and action limits for establishment quality control personnel. These systems operate under Agency oversight with an emphasis on timely and accurate recordkeeping and the necessity for appropriate action to be taken by an establishment when a limit set forth in an approved system is met or exceeded. However, over the last 10 years the number of establishments with active TQC Systems has declined from a high of around 500 (approximately 8% of all establishments) to the present 351 participating establishments (approximately 5% of all establishments). USDA experience has shown that a voluntary approach to HACCP would provide little assurance that a major portion of meat and poultry products had been produced under controls designed to minimize food safety hazards.0

Mandatory HACCP Regulation With Exemption for Small Businesses

Under this alternative, FSIS would mandate HACCP, but also provide an exemption for some category of small businesses as was done with nutrition labeling. While this final regulatory impact analysis does develop very specific definitions for small and very small establishments, the following discussion of comments uses the term ''small'' in a generic sense because many of the comments address small establishments or small businesses without defining these terms. There was a mix of public comments on whether or not HACCP should be mandatory for small businesses.

Comments supporting an exemption from HACCP for small establishments noted that many owner-operators of small establishments oversee the entire operation on a daily basis and can pay closer attention to procedures than can a large establishment. Similar comments pointed out that small establishments pose a minimal potential public health hazard because of the simplicity of their operations, the slow pace of operations, and the small number of potentially affected customers. Other comments pointed out that they sell their product to family, friends and neighbors and that type of market provides the greatest incentive for producing safe product.

Some commenters opposing an exemption did not want to create a two-tiered system. Others opposing an exemption for small establishments would require HACCP for everyone while easing the burden through flexibility of implementation. Several of the commenters opposing any type of exemption from HACCP identified themselves as owners of small establishments. One commenter noted that just because small businesses produce only 2 percent of the product does not mean they are responsible for only 2 percent of the foodborne illness attributable to meat and poultry.

The Agency used the evaluative factors presented above to consider the application of the rule to small establishments. Since major goals in implementing HACCP are to improve processing controls and establishment performance across *all* of industry (factor one) as a means to achieve foodborne illness reduction (factor two), the option to exempt establishments that perform the least process control is inherently flawed. USDA inspection experience shows that some of the small establishments which would be exempted under this option have particular difficulties maintaining control over their processing system.

While it is true that small establishments produce a minimal amount of the total meat and poultry supply, they do produce a full range of products, including those most frequently associated with foodborne illness from the meat and poultry supply.

This option also fails on factor three—provide more effective inspection. Two different inspection systems would be needed: one risk-based system to inspect HACCP establishments with good processing controls; the other to provide resource intensive coverage for establishments that largely do not. If the number of small establishments were to increase, more inspection resources would be required.

For these reasons, the final rule does not include an exemption for small businesses. However, the Agency has made significant changes to ease the burden on small business, including basing microbial sampling programs on production volume and deferring implementation of mandatory HACCP for small and very small businesses as defined in Section V.

Mandatory HACCP Regulation Only for Ready-to-Eat Products

This option would mandate HACCP only for establishments that prepare ready-to-eat meat and poultry products, but not for establishments that produce raw products. However, this decision would leave the public without adequate protection from pathogenic microorganisms clearly associated with product marketed in raw form. Very little reduction in the most frequent causes of foodborne illness (factor two) could be anticipated from this approach.

Government inspection costs would continue to increase to provide traditional resource-intensive inspection for slaughtering and allied processing establishments that would not be subject to mandatory HACCP. Since most of the unsolved problems with pathogenic microorganisms are associated with raw product and not with those products that would be the subject of this HACCP option, this is an especially inappropriate regulatory approach.

Modified HACCP—Recording Deviations and Responses Only

A final alternative considered would be to mandate HACCP, modified to eliminate the record keeping burden to the inspected industry, especially small establishments. Specifically, this option would modify the HACCP record-keeping principles so that instead of demanding continuous records at critical control points, companies would need to record only deviations from critical limits and the response to them. This would mean that HACCP-controlled operations would not generate continuous monitoring data to reflect the operation at critical control points, but would only record data when deviations occurred. This arrangement eliminates the continuous picture of establishment operations which is the underpinning of factor three—make inspection more effective.

Such an approach would substantially reduce the paperwork burdens associated with mandatory HACCP as recommended by NACMCF and recognized by CODEX. However, it would also seriously compromise the usefulness of HACCP as a means to make inspection more effective and avoid program cost increases. Regulatory officials need to have a system which can be reviewed in its entirety, so that a comprehensive picture of the process is available, not just the truncated version which grows out of recording deviations.

E. Comments on Analysis of Regulatory Alternatives

There were several general comments related to either the alternatives discussed in the proposed rule or the level of analysis conducted. There were comments noting that FSIS did not quantify the costs and benefits of the regulatory alternatives. Similar comments suggested that FSIS should have determined cost-benefit ratios for the processed food industry or for ready-to-eat products or for small businesses.

Generating quantitative benefit estimates for different types of products or different industry sectors would be very difficult. The estimates for foodborne illness attributable to meat and poultry have not been broken down by industry sector or type of product. There are no existing estimates for the portion of foodborne illness attributable to meat versus poultry or raw product versus cooked or partially cooked product.

Production volume can not be used as an indicator of potential benefits. Foodborne illness is not proportionally related to production volume because pathogen levels vary significantly by type of product. As noted above, a commenter also pointed out that just because small businesses account for only 2 percent of production does not mean that small businesses account for only 2 percent of foodborne illness.

On the cost side, the estimates are, for the most part, based on industry averages. In reality, costs will vary by industry sector based on the hazards presented and the existing presence of process control. Thus, in response to a comment that suggests that few benefits are available from changing the process for the manufacture of processed foods which are now produced under a zero pathogen standard, the Department would suggest that the costs for implementing HACCP for these products will also be low. Many ready-to-eat products such as cooked patties and roast beef are presently produced under comprehensive process control regulations.

One comment suggested that FSIS consider mandatory HACCP for only firms that produce raw meat and poultry products because that sector of the industry generates most of the problems

and would provide the greatest pathogen reduction benefits per dollar of cost expended. The same commenter found it odd that the Agency did include an alternative for mandatory HACCP for only ready-to-eat products after acknowledging that most of the unsolved problems with pathogenic microorganisms are associated with raw meat and poultry products, rather than ready-to-eat products. In the above discussion of regulatory alternatives, it was noted that mandatory HACCP for only ready-to-eat products is an especially inappropriate regulatory approach. In contrast, a raw product option appears attractive since most of the unsolved problems with pathogenic microorganisms are associated with raw product. Most establishments handle raw product ingredients or prepare a finished raw product. Most of the cost of this rule is associated with controlling the safety hazards of raw product production. Extending the rule to cover all production adds little cost while allowing a single inspection approach, avoiding confusion where raw product production ends and ready-to-eat production begins, and assuring that the potential hazard of recontaminating ready-to-eat product by contact with raw ingredients is always covered by comprehensive HACCP programs.

Other comments noted that FSIS did not analyze an option that accounted for the savings associated with streamlining and modernizing the inspection system or that FSIS should revise the cost-benefit analysis to consider the savings from eliminating the current inspection program. The savings referred to will be used to focus on food safety risks that need more coverage.

III. Summary of Impacts

A. Introduction

This section provides a summary of the costs and benefits that will be discussed in detail in Sections IV and V. The benefits analysis in Section IV and this summary discuss benefits in terms of the reduction in the cost of foodborne illness that results from reductions in pathogen levels. There are other public health benefits beyond the reduction of foodborne illness due to pathogenic bacteria. HACCP systems will also provide increased public protection from risks posed by chemical and physical hazards. There are also benefits beyond public health benefits. As discussed in Section I, the SOP and HACCP requirements have social benefits that derive from the capacity to reallocate inspection resources to other activities where the payoff in terms of reducing the risk of foodborne illness may be greater.

The February 1995 proposal and the subsequent public comment recognized that the HACCP/Pathogen Reduction regulations would also generate benefits for meat and poultry processors. For example, a commenter at a public hearing provided confirmation that the insurance industry is aware of HACCP and has offered reduced liability insurance for firms with improved food safety controls. Other comments noted that improved production efficiency has always been associated with improved process control. Increased customer confidence can also be a benefit to the extent that it has a positive influence on demand.

The benefits analysis in the preliminary RIA noted that benefits also accrue through the reduction of operating costs like the cost of product recalls or the cost of settling product liability claims. Other operating costs include the loss of establishment production due to suspensions for sanitation problems that could be reduced by improved process control, premiums for product liability insurance, loss of product reputation, and reduced demand when a foodborne illness outbreak is publicized identifying a product or company.

The cost analysis in Section V addresses two types of costs associated with this rule. There are the predictable costs associated with requirements directing all establishments or a specific category of establishments to take a well-defined action. Examples include the requirements to develop SOP's and HACCP plans or the requirement to have access to a HACCP-trained individual. This final RIA provides point estimates for all predictable costs. There are also potential costs that may impact some establishments because of current establishment-specific situations. This analysis provides a range of potential costs developed from two different scenarios of possible establishment responses to new pathogen standards.

This summary compares both types of costs with the potential public health benefits related to pathogen reduction, recognizing that there are other potential benefits. The discussion in Section V notes how this rule will set new requirements and also improve compliance with existing requirements. Some of the potential costs discussed in Section V are costs associated with improved compliance with existing standards and should not necessarily be considered costs of this rulemaking.

Public comments demonstrate that the controversy in this rulemaking derives not from the benefit cost ratio itself, which is very favorable, but from the fact that the processors will bear most of the costs while the public, in general, will experience the benefits. The public includes both the consumers of meat and poultry and those who do not consume meat or poultry but who bear the costs of illness in the society. Another area of controversy arises from the lack of proof that the estimated benefits will result from the promulgation of the rule. These doubts are particularly troublesome to those who would have to make resource investments under the rule while benefits largely accrue to others. This is, of course, the standard controversy facing government regulators. The essence of government regulation is that there is a situation where the public undergoes unacceptable risk because the current distribution of costs and benefits is unlikely to change without government intervention. This rule represents the Department's belief that the food safety risks being borne by the public are unacceptable, that they can be reduced through the use of readily available current technologies, and that the uncertainties involved in just how much risks can be reduced should not prevent the Department from making its best effort to reduce the risks.

B. Net Benefit Analysis

Because costs and benefits accrue at different rates over different time periods, to compare costs and benefits it is necessary to examine present value estimates for both cost and benefit streams. To make these comparisons, both the preliminary analysis and this final RIA use a 20-year time period. The present values for costs and benefits are based on a discount rate of 7 percent, the current standard recommended by the Office of Management and Budget.

As discussed above, the cost analysis (Section V) addresses two types of costs. FSIS was able to develop point estimates for the direct costs of complying with the requirements outlined in the rule that all establishments must meet. These predictable costs include the costs of developing and operating HACCP plans and SOP's and the costs of required recordkeeping. There are also potential costs for establishments that may have to purchase new equipment, or modify their production practices to meet the pathogen reduction performance standards for *Salmonella,* or actually implement *Salmonella* testing programs to assure compliance with the new standards. The cost analysis develops a range of cost estimates for these potential costs.

The estimated annual industry costs (not discounted) are summarized in Table 2. These annual costs vary over the first four years as the new HACCP-based program is undergoing its implementation phase. After the initial four years, the recurring costs are estimated at a constant $99.6 to $119.8 million per year. The present value of all industry costs summarized in Table 2 for the 20-year time period is $968 to $1,156 million as shown earlier in Table 1. This total of $968 to $1,156 million ($0.97 to $1.16 billion) is the total industry cost for the rule as shown in Table 3.

TABLE 2.—SUMMARY OF ANNUAL INDUSTRY COSTS—ALL REQUIREMENTS

[$ Thousands]

Cost Category	Year 1	Year 2	Year 3	Year 4	Year 5+
I. Sanitation SOP's:					
Plans and Training	2,992				
Observation and Recording	8,345	16,691	16,691	16,691	16,691
II. *E. coli* Sampling:					
Plans and Training	2,627				
Collection and Analysis	8,716	16,122	16,122	16,122	16,122
Record Review	406	752	752	752	752
III. Compliance with *Salmonella* Standards		5,472–16,899	5,353–25,753	5,811–25,956	5,811–26,079
Compliance with Generic *E. coli* Criteria		(1)	(1)	(1)	(1)
IV. HACCP:					
Plan Development		3,769	27,755	35,464	
Annual Plan Reassessment			69	448	1,179
Initial Training		1,270	8,284	18,435	
Recurring Training		64	542	1,877	2,799
Recordkeeping (Recording, Reviewing and Storing Data)		3,050	18,479	42,478	54,097
V. Additional Overtime		189	837	1,711	2,125
Total	23,086	47,379–58,806	94,884–115,284	139,789–159,934	99,576–119,844

[1] Not Separately Estimated.

TABLE 3.— PRESENT VALUE OF 20-YEAR COSTS AND BENEFITS

[$ Billions]

Effectiveness in reducing pathogens in the manufacturing sector (percent)	Public health benefits		Industry costs
	Low	High	
10	0.71	2.66	0.97–1.16
20	1.43	5.32	0.97–1.16
30	2.14	7.98	0.97–1.16
40	2.85	10.64	0.97–1.16
50	3.57	13.30	0.97–1.16
60	4.28	15.96	0.97–1.16
70	4.99	18.61	0.97–1.16
80	5.71	21.27	0.97–1.16
90	6.42	23.93	0.97–1.16
100	7.13	26.59	0.97–1.16

Note: Analysis assumes zero benefits until year 5. All elements of the HACCP-based program will be in place 42 months after publication of the final rule.

The public health benefits of this rule are discussed in detail in Section IV. The benefits are based on reducing the risk of foodborne illness due to *Campylobacter jejuni/coli, Escherichia coli* 0157:H7, *Listeria monocytogenes* and *Salmonella*. Section IV concludes that these four pathogens are the cause of 1.4 to 4.2 million cases of foodborne illness per year. FSIS has estimated that 90 percent of these cases are caused by contamination occurring at the manufacturing stage that can be addressed by improved process control. This addressable foodborne illness costs society from $0.99 to $3.69 billion, annually. The high and low range occurs because of the current uncertainty in the estimates of the number of cases of foodborne illness and death attributable to the four pathogens. Being without the knowledge to predict the effectiveness of the requirements in the rule to reduce foodborne illness, the Department has calculated projected health benefits for a range of effectiveness levels, where effectiveness refers to the percentage of pathogens eliminated at the manufacturing stage. The link between effectiveness and health benefits is the proportionate reduction assumption which is explained in Section IV. Because of the wide range in estimates for the cost of foodborne illness, each effectiveness level will have a low and high estimate for public health benefits. These estimates of public health benefits are shown in Table 2, as the present value of a 20-year benefit stream.

The analysis assumes that benefits will begin to accrue in year five. The five year lag leads to conservative benefit estimates since the new HACCP-based inspection program will be fully implemented in 42 months, and benefits should accrue during those 42 months as well as in the 1½ years that follow. Limiting the benefit estimates to four pathogens also leads to conservative cost estimates. To the extent that the proportionate reduction estimate may overestimate benefits, these other factors provide conservative balance.

Net benefits exist for every cost and benefit combination illustrated in Table 2 except for the case of 10 percent effectiveness using the low benefit estimate. If the low benefit estimate is correct, the new HACCP-based regulatory program would have to reduce pathogens by 14 to 17 percent to cover the projected 20-year industry costs of $968 to $1,156 million. For the high benefit estimate net benefits begin to occur at an effectiveness level of 4 to 5 percent.

The costs summarized in Tables 1 and 2 have not been reduced to account for firms that already have existing HACCP programs. FSIS does not have a good estimate of the number of such firms.

C. Impact on ''Smaller'' Businesses

The final rule provides regulatory flexibility for smaller firms consistent with the Regulatory Flexibility Act. For the slaughter facilities, the generic *E. coli* sampling requirements vary depending on the number of birds or animals slaughtered annually. This will significantly reduce the microbial